The University of Alberta
Printing Department
Edmonton, Alberta

THE UNIVERSITY OF ALBERTA

UPTAKE OF PICLORAM BY ALFALFA AND BARLEY ROOTS

by

IAN N. MORRISON

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

OF MASTER OF SCIENCE

IN

WEED SCIENCE

DEPARTMENT OF PLANT SCIENCE

EDMONTON, ALBERTA

SPRING, 1973

ABSTRACT

Uptake of ^{14}C-picloram, a potent auxinic herbicide, from culture solution by intact roots of alfalfa and barley seedlings was investigated using radiotracer techniques.

Time-course studies indicated that alfalfa and barley have similar absorption patterns consisting of rapid initial uptake, lasting for 1 to 2 hr, followed by continuing absorption occurring at a lower, nearly constant rate for the remainder of a 12-hour treatment period. At pH values less than the pK of picloram, uptake of the acid was substantially increased.

Initial uptake was not significantly reduced by respiratory inhibitors, whereas continuing absorption was markedly depressed. Q_{10} values for the first hour of uptake were 1.4 for alfalfa and 1.0 for barley, whereas for periods exceeding 2 hr, Q_{10} values were consistently greater than 2.0. Evidently, uptake during the initial phase of entry is governed by passive processes, while continuing absorption is controlled, at least in part, by active mechanisms.

Rapid release of much of the picloram initially taken up by alfalfa and barley roots to unlabelled nutrient solution suggests that diffusion is the principal means of passive absorption. Limited uptake under dark conditions and enhanced uptake in the presence of sucrose indicate that active absorption is dependent on energy derived from carbohydrates and other energy substrates formed during photosynthesis. Externally applied ATP, however, did not stimulate uptake significantly.

The results of inhibitor studies suggest that picloram absorption by alfalfa plants is more dependent on a supply of metabolic energy than is uptake by barley plants. In most instances, total accumulation of picloram was markedly greater in alfalfa plants than in barley plants of nearly equivalent mass, which may partially account for the difference in susceptibility of alfalfa (sensitive) and barley (tolerant) to the herbicide.

Decidedly, uptake and transport of picloram involves both a strong physical diffusion component as well as associated metabolic processes. The relative importance of the two means of entry and movement within plants apparently varies between species.

ACKNOWLEDGEMENTS

I wish to express gratitude to Dr. W. H. Vanden Born who showed enthusiasm and offered invaluable counsel, comments and criticism during the course of this study.

Gratitude is also expressed to Mr. R. J. Schraa for his capable technical assistance, particularly in the preparation of photographs, and to Dr. P. R. Gorham for his invaluable advice on microautoradiographic techniques.

Thanks are due to Dow Chemical Company for supplying radiolabelled and analytical grade picloram for this investigation.

Special thanks are extended to my wife, Joanne, and to my parents who were always most cooperative.

Financial assistance provided by the National Research Council of Canada is gratefully acknowledged.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Figure | Page

INTRODUCTION

The characteristics of absorption and translocation of soil-applied herbicides vary considerably and are dependent on inherent factors of the plant as well as on certain conditions of the environment. Current evidence indicates that both passive and active processes are involved in the uptake and movement of a number of synthetic growth regulators by plant roots (45, 65, 73, 110). Passive processes occur spontaneously whereas active processes require the expenditure of metabolic energy.

Picloram is a potent auxinic herbicide which has proven to be very effective for the control of deep-rooted perennial weeds. In general, broad-leaf plants are quite susceptible to the chemical whereas grasses are resistant. Picloram is rapidly translocated following either root or foliar application (5, 24, 77, 84). It is not readily metabolized in plants and is not subject to rapid degradation by soil microorganisms (39, 52, 62, 84, 86).

In many respects the effects of picloram on plants resemble the effects of 2,4-D. Herbicidal dosages of both compounds induce similar growth aberrations including distortion of stems, inhibition of apical growth, and formation of adventitious roots (24, 28, 39, 52). Although the primary mode of action is unknown, various investigators have reported that picloram causes disruption of chloroplast structure, loosening of cell walls, increased RNA and protein synthesis, and uncoupling of the processes involved in the formation of high energy phosphorylated compounds essential for normal cellular metabolism (3, 13, 24, 87).

Much of what is known about the mechanisms involved in picloram absorption has been obtained from studies conducted with excised roots and various other plant tissues (6, 78, 86, 97). Accordingly, a study of the factors which affect absorption and translocation of picloram by intact roots of alfalfa (susceptible) and barley (resistant) plants was undertaken. The objectives of the study were to characterize the general features of picloram absorption from culture solution by two species of differing susceptibility to the herbicide, and to determine to what extent passive and/or active mechanisms are involved in the uptake of picloram by intact roots.

LITERATURE REVIEW

The mechanisms involved in the absorption and long-distance transport of solutes by roots have been extensively studied for several decades and the topic has been reviewed in a number of articles (2, 11, 26, 53, 56, 58, 69, 96). Much evidence has accumulated confirming that, in addition to a number of passive processes, active processes requiring the expenditure of metabolic energy play an important role in the absorption and subsequent translocation of solutes by plant roots. Although much effort has been directed towards the study of mechanisms of solute absorption and transport, an unequivocal description of the process has not been forthcoming.

A number of physical mechanisms are involved in the uptake of solutes by roots, including adsorption, diffusion, and mass flow. These are considered passive processes, for they occur spontaneously and do not require the expenditure of energy. Active processes, on the other hand, must utilize energy provided by aerobically respiring cells. Both active and passive processes are usually explained in terms of structural characteristics of the root, although relationships between root structure and solute absorption are still not clearly understood.

Structural characteristics of roots

Roots are structurally complex organs specialized for absorbing water and nutrients from the soil. The outermost layer is the epidermis, consisting of compact, thin-walled cells some of which undergo morphological transformations to become root hairs. The epidermis is free of

intercellular spaces. The cortex lies beneath the epidermis and is composed of loosely arranged parenchyma cells arranged in partially disturbed concentric circles and interspersed with numerous intercellular spaces. Separating the central cylinder or stele from the cortical tissue is the endodermis.

The endodermis forms a sheath of one layer of cells around the central cylinder and is, like the epidermis, completely free of intercellular spaces. In the primary state the endodermal wall contains suberin in a band-like structure extending completely around the cell within the radial and transverse walls, thereby forming a ring around each cell. The interconnecting system of rings running from cell to cell throughout the entire endodermal sheath is known as the Casparian strip. With maturity, the endodermis develops thick secondary walls which typically consist of a suberin lamella covered by layers of lignified cellulose. The formation of the secondary walls may be delayed in the endodermal cells opposite the xylem. Such thin-walled cells in an otherwise thick-walled endodermis are called passage cells. The suberized portions of the endodermis are considered to be impermeable to the passage of water and solutes into the stele by diffusion or mass flow; the significance of this will be discussed later.

The center of the stele consists of a vascular core with primary xylem as the central tissue with strands of phloem alternating with xylem ridges. If xylem does not differentiate in the center of the root, a pith consisting of parenchyma or sclerenchyma cells is present. Frequently, the roots of monocotyledonous species contain a pith encircled by a ring of xylem vessels.

Radial movement of solutes through roots may occur either in the living (symplast) or non-living (apoplast) portion of a root. According to Salisbury and Ross (83), these terms were introduced by Münch in 1932, with symplast designated as the sum total of interconnecting living protoplast of the plant, and apoplast referring to the remaining system of non-living, interconnected cell walls and intercellular spaces including the water-filled (or air-filled) xylem elements. Most probably, solutes migrate over long distances first through the free space and after having overcome a physiological barrier, presumably the plasmalemma of the cortical cells, continue to move through the symplast.

The free space has been described by Laties (53) as that part of a plant tissue which is in free diffusion communication with the environment without permeation barriers. Quite probably the free space, or more specifically the apparent free space of roots, consists of cell walls and the intercellular spaces of the epidermis and cortex, and is approximately but not exactly, equal to the apoplast. The apparent free space has been further subdivided into water free space (WFS) and Donnan free space (DFS) (11, 69). The WFS is, in essence, an extension of the environment into which solutes can move in accord with diffusion expectations such that they attain the same concentration as in the external solution. The DFS, however, contains a high concentration of fixed negative charges attributed to the unmethylated carboxyl groups of various pectic substances in cell walls which affect the final concentration of solutes in that region.

Current evidence indicates that the barrier to free diffusion of

ions into root cells is located at the plasmalemma (38, 69, 71, 83). This, in turn, means that the principal barrier to diffusion and mass flow of water and solutes through the free space is in all likelihood the Casparian strip of the endodermis (4, 23, 83, 112). Under these conditions, passive movement of substances from the cortex to the stele of young roots would occur only by Donnan exchange phenomena, changes in permeability of the plasmalemma or as a result of breaks in the Casparian strip or endodermis.

Dumbroff and Peirson (23) have reported that a significant number of sites are available that may favor passive transport of ions into the root xylem. As branch initials develop, suberin deposition apparently does not keep pace with cell division or cell growth. Many branches observed at this stage appeared to show direct pathways for mass flow along the cell walls from parent cortex to parent xylem.

Although some investigators have maintained that the only course of movement of solutes through mature endodermis is via the passage cells (69), more recent evidence indicates that these cells make little more than a minor contribution to the transport of water and solutes into the stele (15). Indeed, protoplasmic connection appears to be maintained between the cortex and the pericycle by plasmodesmata throughout progressive development of the endodermis (15, 16, 17). Plasmodesmata are now considered to be the principal channels of solute movement into the stele.

Metabolic coupling of active transport

Energy for active solute transport in plants may be derived either

directly from high-energy phosphorylated compounds such as adenosine triphosphate (ATP) or by linkage to electron transport processes. Some investigators have concluded that uptake of anions is coupled directly to the electron transport system and that cations follow passively along an electrical potential gradient (34, 58). From studies with giant algal cells, MacRobbie (61) suggested that anion uptake was linked to the electron transport chain whereas cation absorption was powered by ATP. Considerable evidence for the role of an adenosine triphosphatase (and therefore ATP) in energy dependent transport of ions has recently been reported (30, 31, 36, 37, 57, 85). Fisher and Hodges (31) and others (30, 57) have isolated a membrane-bound, monovalent ion-stimulated ATP-ase in oat and corn roots which appears to have sufficient activity to account for observed rates of ion transport.

The actual mechanism(s) of active solute transfer across the plasmalemma and subsequent long distance transport through the symplast to vascular tissue is unknown. A number of theories, mostly pertaining to transport of mineral nutrient ions, have been proposed which could conceivably be applied to the uptake of organic compounds as well.

Transport across cell membranes

A widely accepted concept of ion transport across the plasmalemma is that a mobile carrier molecule binds the ion at the external interface of a membrane by adsorption, exchange adsorption, or chemical combination, and then migrates to the opposite interface as an ion-complex whereupon the ion is released into the internal solution

(27, 96). Accumulation of ions behind a physiological barrier by means of carriers is considered to be an active process dependent on metabolic energy. The existence of ion carriers in biology has remained largely hypothetical although quite recently certain naturally-occurring substances have been directly implicated in ion transport across membranes (55).

According to the anion-respiration theory developed by Lundegardh (58), a direct relation exists between salt repiration and ion transport across membranes. Anions are assumed to be accumulated by moving in the opposite direction to that of the electrons moving along the cytochrome chain of the oxidative phosphorylation process. Cations are thought to be absorbed passively along 'adsorption tracks' under the influence of an electrical gradient created by the absorption of anions.

Hall (38) has recently proposed that the mechanism of ion transport in root cells involves invagination of the plasmalemma and the uptake of ions in small vesicles which later dissolve releasing their contents to the cytoplasm. An active process involving ATP which is utilized by an active ATP-ase system located in the plasmalemma and vesicular membrane is suggested to regulate this mechanism.

Although adsorption *per se* is a physical phenomenon, some investigators have speculated that metabolic energy may be required to maintain the integrity of adsorptive sites on membranes and other root surfaces (22, 90, 96, 110). Sutcliffe (96) suggested that

continuous uptake by adsorption can be linked with metabolism either through synthesis of new adsorptive sites during growth, or by vacation of sites as a result of metabolic utilization of the adsorbed compound. Studies conducted by Shone and Barber (90) have indicated the presence of metabolically maintained positive sites in barley roots which would facilitate anion absorption.

Radial transport of solutes in roots

According to the most often cited Crafts-Broyer Theory (19, 27, 83) of radial salt transport in shoots, salt is actively accumulated in the cytoplasm of cortical cells and subsequently moves inward principally by diffusion to the stele where it leaks out of the stelar parenchyma cells into the xylem. In this "one pump" model, transport across the plasmalemma is considered to be the only metabolically mediated event; the subsequent movement of ions to the vascular tissue is deemed to be passive. The observations of Laties and Budd (54) that the cells of the stele are both leaky and ineffective in salt absorption strengthen this theory. Some controversy exists, however, whether salts passively leak into the xylem or whether they are actively secreted into the vascular tissue (111, 112).

Some investigators have proposed that the endodermis may actively secrete salt into the stele (2, 96) whereas others have suggested that stelar parenchyma cells are involved in active secretion of salt into the xylem (53, 111). A view that two active mechanisms of transport are involved in ion transport to the shoot has recently been expounded by Pitman (71). In his "two pump" hypothesis a second active transport

process, additional to active transfer of ions across the plasma membrane of the cortical cells, is purported to actively secrete ions into the xylem.

Localization of solutes in roots

Microautoradiographic techniques have been used with some success to elucidate the transverse pathway of solute movement through roots. From studies showing heavy accumulation of ^{59}Fe in the cell layers adjacent to the endodermis in pea roots treated for 3 hr in a $^{59}FeCl_3$ solution, Branton and Jacobson (10) concluded that iron must accumulate in root parenchyma cells before release to the xylem occurs. Biddulph (7) speculated that ^{45}Ca and ^{35}S follow different routes through cortical tissue of intact bean roots. After one hour and fifteen minutes ^{45}Ca was most evident in cell walls, whereas ^{35}S was more evenly distributed throughout the cells. Furthermore, the endodermis did not appear to be a barrier to inward movement of ^{45}Ca but significantly impeded movement of ^{35}S into the stele.

Strang and Rogers (94) concluded that radial movement of diuron in cotton and soybean roots is limited to the apoplast, since sections of roots treated with ^{14}C-diuron showed a general diffuse labelling throughout the walls of the cortical cells. Donaldson (22) observed a similar distribution of radioactivity in barley roots treated with either ^{14}C-monuron or ^{14}C-diuron. High concentrations of root-absorbed ^{14}C-2,4-D, on the other hand, appeared to be adsorbed to the outer edge of the epidermis while very little accumulated in the rest of the tissue. Similarly, Strang and Rogers (95) showed that ^{14}C-

trifluralin was tenaciously adsorbed or bound to the epidermis of both cotton and soybean roots. Breaks in the epidermis appeared to facilitate ^{14}C-trifluralin entry into the cortical tissue, however, the endodermis appeared to act as a barrier to movement of the herbicide into the stele.

Uptake and transport of growth regulators and herbicides

In comparison with investigations on nutrient ion absorption, the uptake of organic molecules by plant tissues has not been studied extensively. The early work of Crafts and his associates (20, 110) provided much information on the relative amounts of absorption and the comparative mobility of many growth regulators in a number of plant species. Since Crafts' utilization of the autoradiographic technique a great many similar investigations concerning root uptake and distribution of herbicides have been undertaken (33, 67, 88, 92, 107). Until recently, however, little attention has been paid to the actual mechanisms of absorption of growth regulators and herbicides by plants or to the factors which affect uptake and transport in plant tissues.

Absorption of a number of growth regulators into plant tissues has been shown to occur in two phases: an initial rapid uptake followed by metabolically mediated continuing absorption at a lower rate (50, 72, 79). Moody *et al.* (65) reported that the uptake of a number of herbicides including atrazine, chlorpropham, linuron and EPTC was initially very rapid, followed by a much slower uptake, the rate of which generally decreased as time increased. A similar study

conducted by Vostral *et al.* (106) showed that uptake of atrazine by roots was rapid during the first 30 minutes, at least tenfold greater than the subsequent uptake which was maintained at a uniform rate for 24 hr.

Physical absorption processes are assumed to be completed relatively rapidly, whereas the period of metabolic absorption can be prolonged. Initial passive absorption is considered to be reversible whereas prolonged metabolic absorption processes are deemed irreversible. Johnson and Bonner (50) and others (22, 73, 82) have shown that a major proportion of an organic compound taken up in the initial phase is readily exchangeable, and that the amount which is exchangeable after longer periods of absorption is approximately equal to the amount initially absorbed. Solutes which move into the WFS by diffusion are readily lost upon transfer of the tissue to water. Those held in the DFS by adsorption are not lost on placing the tissue in water, but can be removed through exchange by placing it in a solution of a similar solute. Tames and Hance (99) and others (22, 91, 110) have suggested that, in some cases, adsorption may be a significant pathway for herbicide uptake by roots.

Factors affecting absorption and translocation

1. pH

The pH of the ambient solution exerts a marked effect on the absorption of many herbicides and growth regulators (22, 45, 79, 97, 110), although the influence of pH on the uptake of some compounds such as monuron is negligible (22). Reduced pH usually results in a greater uptake. According to Yamaguchi (110) and others (22, 45),

absorption of organic compounds is greatest when the molecules are in the undissociated form, presumably due to the fact that the uncharged molecules are more readily able to approach the negatively charged cellulosic and membrane surfaces.

2. External concentration

If diffusion and/or mass flow are the only absorptive mechanisms involved, uptake of a compound tends to be directly proportional to the concentration in the external solution. Lack of saturation effects in a concentration series is characteristic of entry by passive processes. However, if adsorption or an active process is the means whereby absorption occurs, the relationship between uptake and external concentration is generally hyperbolic in nature.

Prasad and Blackman (73) showed that initial rapid uptake of dalapon by roots of *Lemna minor* was linearly related to external concentration. Similarly, Vostral *et al.* (106) recently demonstrated that atrazine uptake by soybeans was essentially linear over a concentration range of 0.02 to 0.08 mM, roughly paralleling water absorption. Donaldson (22) reported that total uptake of 2,4-D by barley roots treated for 1/2 and 4 hr leveled off as external 2,4-D concentration was increased, whereas total accumulation of monuron remained linearly proportional to the external concentration. Follow-up experiments revealed that 2,4-D was apparently tenaciously adsorbed onto the root surface.

Accumulation of solutes against a concentration gradient is presumed to require metabolic energy and is often used as a criterion for active root absorption. Many plant physiologists have recorded

accumulation of auxinic substances in plant tissues in excess of ambient concentrations (50, 72, 110). Similar results have been reported for a number of herbicides (65, 84).

Accumulation ratios, described as the concentration within a root/concentration in the external solution, have been derived by Moody *et al.* (64) for uptake of several herbicides by soybean roots. Their results showed a range from 1.73 for amiben to 4.95 for linuron after only 1 hr, implying rapid metabolic absorption. Nashed and Ilnicki (68), however, concluded that the amount of linuron absorbed by corn and soybean plants was equal to that contained in the solution taken up by the plants, and that uptake appeared to be passive.

Shone and Wood (91) reported that the concentration of simazine in barley roots, measured on a fresh-weight basis, reached a value two times that in the ambient solution within two hours. However, the concentration of simazine in the xylem sap did not attain that of the uptake solution. Part of the accumulation was attributed to a physical adsorption of the compound on the roots.

3. Metabolic inhibitors

Marked reductions in uptake of growth regulators and certain herbicides by plant tissues in the presence of metabolic inhibitors have been clearly demonstrated (14, 45, 50, 73, 79, 86, 98, 110). The initial phase of uptake has been shown to be less sensitive to inhibitors than is subsequent active absorption. Prasad and Blackman (73), for example, reported that the initial uptake of dalapon by roots of *Lemna minor* was relatively insensitive to 2,4-dinitrophenol, whereas the prolonged absorption was retarded or suppressed entirely

by 2,4-dinitrophenol, sodium azide and sodium arsenite.

Reduction of active solute absorption generally occurs over the same range of inhibitor concentrations that is required to suppress respiration (93, 96). Certain inhibitors of protein synthesis (e.g. chloramphenicol) however, may reduce salt absorption without any appreciable effect on respiration (96). At lower concentrations, some 'inhibitory' compounds have been shown to stimulate respiration and, at the same time, increase solute absorption (14, 40, 45).

Translocation of some herbicides can be stimulated by the application of certain metabolic inhibitors at concentrations above those necessary to reduce respiration (100, 110). Taylor and Warren (100) concluded that the major physiological effect resulting in increased herbicide translocation following inhibitor application is an alteration of the semi-permeability status of membranes involved in the retention process, and the greater the tendency of a compound to be bound, the more pronounced the increase in translocation will be following treatment with a metabolic inhibitor.

Shone and Wood (91) have recently reported that 2,4-dinitrophenol and sodium azide had a considerable inhibiting effect on both the total quantity of simazine taken up by intact barley roots and on the amount that was transported to the shoots. Since uptake of simazine *per se* is considered to occur by passive processes, the reduced uptake was attributed to an observed decrease in the rate of transpiration.

4. Temperature

In general, an increase in temperature, within physiological

limits, results in increased absorption of compounds by plant tissues as well as enhanced root-to-shoot movement in intact plants (14, 45, 50, 65, 66, 76, 79, 91, 106).

Total solute absorption, either by plant tissues or by whole plants, can be separated into two components. One of these has a temperature coefficient (Q_{10}) of 1.2 to 1.3, indicating that purely physical processes such as diffusion are involved, and the other has a Q_{10} or 2 higher, indicating that metabolic energy is required. Temperature coefficients greater than 2 have been recorded for the continuing uptake of a number of growth regulators into various plant tissues (8, 14, 79, 86). The results of a study conducted by Moody *et al.* (65), however, show that Q_{10} values for the absorption of several herbicides by excised soybean roots were always less than 1.85.

Vostral *et al.* (106) have reported recently that accumulation of atrazine in the roots of intact plants was little affected by lowered temperature, while translocation from the roots to the leaves was markedly impeded. Similar results have been obtained by Shone and Wood (91) who reported that lowering the temperature of the uptake solution resulted in a considerable decrease in the total quantity of herbicide absorbed coupled with a corresponding reduction in the amount of water transpired. Moody *et al.* (65) concluded from their studies, however, that in most instances transpiration had little or no direct effect on the rate of herbicide uptake and that root temperature *per se* was much more important than transpiration in affecting the rate of uptake of herbicides. Although transpiration was shown to increase as temperature increased, herbicide uptake and

transpiration were considered to be unrelated.

5. Light

The effects of light on uptake and translocation of growth regulators and herbicides are mainly indirect. Light has effects on the structure of leaves and the condition of stomata which may indirectly affect solute absorption and movement through enhanced transpiration (96). Shone and Wood (91), however, have recently concluded that, although reduced uptake of simazine by barley plants under low illumination is attributable mainly to a decrease in the amount of water transpired, small but significant differences between the relative movement of the herbicide and water occur when light intensity is varied.

Energy required for solute absorption is considered to be supplied either through reductive mechanisms associated with photosynthesis or through utilization of carbohydrate reserves in the process of oxidative phosphorylation. Yamaguchi (110) has demonstrated that by depleting food reserves in bean plants kept in darkness for 5 days, 2,4-D absorption was considerably reduced. Other studies have revealed that when excised roots, non-green plant tissues or photosynthetic organisms in the dark are depleted of carbohydrate reserves, solute absorption can be stimulated by an external supply of sugar (96, 86).

6. Anoxia

The metabolic aspect of absorption of growth regulators has been investigated by continuous bubbling of nitrogen through culture

solutions as a means of inducing anoxia (14, 72, 110). Considerable reduction in uptake of auxinic substances has occurred when oxygen is excluded from plant tissues in this manner. Poole and Thimann (72) pointed out that initial uptake of IAA by *Avena* coleoptile sections is not significantly reduced in purified nitrogen, although the subsequent rate is about one-half that in air.

Picloram absorption and transport

The uptake and translocation of picloram by intact plants as well as absorption by various excised tissues appear to involve a strong physical diffusion component as well as associated metabolic processes. The relative importance of the two means of entry and movement within plants apparently varies between species.

Sharma (86) reported that Q_{10} values for uptake of picloram by excised plant tissues fell between those normally encountered for purely passive processes (Q_{10} 1.0 to 1.3) and active processes (Q_{10} 2.0 to 3.0). Isensee *et al.* (45) concluded on the basis of time course studies and temperature experiments that metabolic activity was more important in soybeans than in oats, although the overall contribution of metabolism in root absorption and translocation of picloram by soybeans was unclear.

The initial phase of picloram absorption from culture solutions by roots of intact plants appears to occur predominantly by passive processes. Isensee *et al.* (45) reported that a linear relationship existed between picloram uptake and the concentration of the external solution over the first thirty to forty minutes of exposure to the

herbicide, suggesting a simple diffusive entry. Over longer absorption periods, however, total accumulation patterns markedly differed for different species; accumulation was concentration dependent for oats but not for soybeans.

Scott and Morris (84) reported that the concentration of picloram in root tissue exceeded the concentration in the external solution, implying that active uptake mechanisms are involved in absorption. Others (45, 86) have demonstrated that metabolic inhibitors caused marked reductions in the amount of picloram absorbed by both excised tissues and intact plants. At the same time, in intact plants dinitrophenol and sodium azide (10^{-6} to 10^{-5} M) stimulated translocation of picloram to the shoots (45). Sharma (86) observed that addition of respiratory substrates to the incubation medium increased the amount of picloram absorbed by excised tissues by 27% while addition of 4×10^{-3} M ATP nearly doubled the uptake. This evidence strongly suggests that energy relationships are intimately involved in the continuing absorption of picloram.

Isensee *et al.* (45) concluded that picloram (pK 4.1) is absorbed preferentially in the undissociated form since uptake by oats and soybeans was markedly reduced when pH of the treatment solution was increased from 3.5 to 4.5. Baur and Bovey (6), however, proposed that uptake is not entirely dependent on the availability of undissociated molecules, since in their studies with potato tuber discs a change of pH from 4.5 to 5.5 reduced picloram absorption by a factor of only 3.2 whereas the availability of the undissociated acid was reduced by a factor of 9.8. Baur and his colleagues (6, 97) have suggested that

the uptake of picloram occurs in part via an unstable accumulation mechanism similar to that proposed by Saunders *et al.* (82) and Venis and Blackman (103, 104) for other auxinic herbicides. The uptake of picloram was related to the availability of quaternary ammonium binding sites provided by membrane phosphatides. Reduced absorption at pH 5.5 was attributed to increased enzymatic degradation of binding sites by an enzyme, phospholipase D, which shows maximum activity at pH 5.5.

MATERIALS AND METHODS

Plant Culture

Alfalfa seeds (*Medicago sativa* L. cv. Ladak) were germinated on moist filter paper in the dark at 25°C. After five days, the seed coats were removed and the seedlings were suspended from perforated aluminum foil with the radicles protruding into half strength Hoagland No. 2 nutrient solution (41). The seedlings were placed in a growth chamber under a 10 hr light/14 hr dark regime. Light intensity at plant level was 13,000 to 15,000 lux from a mixture of fluorescent and incandescent lamps. Day temperature was 21°C while night temperature was 20°C. Relative humidity was maintained between 70 and 80 per cent. After the primary leaf emerged, healthy plants were individually transferred to 20 ml vials covered with aluminum foil. The nutrient solution was replenished every two to three days. When the third trifoliolate leaf had fully expanded, uniform plants (three weeks old) were selected for treatment. Roots and shoots weighed approximately 160 and 300 mg, respectively.

Barley seeds (*Hordeum vulgare* L. cv. Parkland) were germinated in moist vermiculite in the dark at 25°C. After six days, the vermiculite was carefully washed from the roots and the seedlings were individually transferred to foil-wrapped vials containing half strength Hoagland No. 1 nutrient solution (41) modified according to the following recipe:

Stock Solutions	Volume/L
1M KH_2PO_4	2 ml
1M KNO_3	2 ml
1M $Ca(NO_3)_2$	3 ml
1M $MgSO_4$	2 ml
Versonal FL	0.1 ml
Micronutrients	1 ml
$Na_2SiO_3.9H_2O$ (101.43 g/L)	1 ml

The seedlings were placed in a growth chamber under conditions identical to those previously described for alfalfa. When the second leaf had fully emerged, uniform plants (two weeks old) were selected for treatment. Roots generally weighed about 250 mg whereas shoots weighed approximately 380 mg.

Experimental Procedures

Unless specified otherwise, the materials and methods described herein applied to all experiments.

The radioactive 4-amino-3,5,6-trichloropicolinic acid (^{14}C-picloram) was labelled in the carboxyl position and had a specific activity of 1.03 mc/mM (4.25 µc/mg). Both the ^{14}C-picloram and the analytical grade picloram (99% pure) used for preparation of unlabelled herbicide solutions were gifts from Dow Chemical Co., Inc. Stock solutions of ^{14}C-picloram were prepared by dissolving the acid in 95% ethanol. Treatment solutions (0.1 µc/10ml) were prepared by adding ^{14}C-picloram stock solution to half strength nutrient solution. The pH of the treatment solutions was adjusted with 0.5 N H_2SO_4 or

0.5 N KOH. In all investigations but one, the initial pH of the treatment solutions was adjusted to 4.5 for alfalfa and 6.8 for barley. Roots were treated in 10 ml of solution except in time course studies in which case they were treated *en masse* in 100 ml.

Each treatment comprised three plants, each of which was considered to be one replicate. Experiments were repeated up to four times and were conducted separately for alfalfa and barley.

Plants were treated in a growth cabinet under the following conditions: light intensity, 12,900 lux; root temperature (water bath), 23°C; air temperature 21±1°C; relative humidity, 55-60 per cent. Other than in time course studies, in which plants were harvested after 1/2, 1, 2, 4, 8, and 12 hr, plants were generally exposed to treatment solution for 4 hr. In metabolic inhibitor studies 2,4-dinitrophenol (DNP), sodium azide (NaN_3), cycloheximide, and chloramphenicol were dissolved directly in the treatment solution. Oligomycin was first dissolved in 95% ethanol. In other experiments adenosine-5-diphosphate, monosodium salt (ADP); adenosine-5-triphosphate, disodium salt (ATP); succinate and sucrose were similarly dissolved directly in the treatment solution.

At the end of a treatment period roots were thoroughly rinsed in tap water and gently blotted dry. For radioassay by liquid scintillation spectrometry, roots and shoots were digested according to a method described by Reid and Hurtt (77). A mixture of 0.2 ml of 60% $HClO_4$ and 0.3 ml of 30% H_2O_2 was added to scintillation vials containing the tissue, after which the vials were tightly sealed

and placed in an oven at 80°C for 2 hr. After the digested tissue cooled to room temperature, 18 ml of scintillation cocktail (1 part 2-ethoxyethanol, 2 parts toluene (v/v), 6 g/L 2,5-diphenyloxazole) were added to each vial. Samples were counted for ten minutes each, either in a Nuclear Chicago Mark I or a Beckman LS-200B liquid scintillation spectrometer. Sample counts were corrected for efficiency by a channels ratio method and expressed as dpm per root or shoot. The sum of the activity in a given root plus that in the corresponding shoot was expressed as total dpm per plant. Data presented in tabular or graphical form are means of three replicates from a single representative experiment.

Autoradiographic investigations were carried out according to the methods described by Crafts and Yamaguchi (21). Plants were mounted on cardboard, rapidly frozen with crushed dry ice, and placed in a freeze-drier for two to three weeks. The freeze-dried plants were covered with Saran wrap and exposed to Kodak Blue Brand medical x-ray film for three weeks. The films were developed in Kodak Liquid X-ray Developer at 20°C for two and one-half minutes and fixed for ten minutes or longer in Kodak fixer.

Microautoradiographic Studies

Roots were treated under the conditions described in the previous section. Autoradiographic localization of ^{14}C-picloram in roots was attempted using methods described by Gorham and his associates for localization of ^{14}C-labelled sugars in various plant tissues (70,101,108). Pieces 4 to 5 mm in length were excised from

treated roots and immediately frozen in an isopentane bath (-150°C) surrounded with liquid nitrogen. Segments, 1 to 2 mm in length, were cut from the frozen pieces with a scalpel and transferred to aluminum foil boats containing degassed paraffin (Fisher Tissuemat, melting point 56.6°C). The foil boats, packed in crushed dry ice, were then transferred to a Virtis 10-PR Preservator containing 20 g of chemical dessicant (phosphorus pentoxide). The frozen pieces were lyophilized for three to five days at -35°C.

The segments were infiltrated for two to three weeks in a vacuum oven and were centered in the paraffin block under an infrared light. Sections 10 μ thick were cut on a rotary microtome. To avoid excessive compression during sectioning, the blocks were chilled in a downdraft from dry ice.

The sections were mounted on Kodak Nuclear Track Plates, Type NTB 2(10 μ emulsion thickness) and exposed for eight weeks at -20°C in light-tight slide boxes. The paraffin was removed from the sections by carefully immersing the plates in re-distilled xylene, after which they were gently dried under a stream of warm air. The microautoradiographs were developed in Kodak D-19 Developer for ten minutes at 20°C, rinsed in running water for ten minutes, fixed in Kodak fixer for four minutes, washed in running water for another thirty minutes and finally dried slowly in a dust-free atmosphere. All dark-room procedures were carried out under a Kodak Safelight Filter No. 2. Sections were examined under a light microscope.

RESULTS

The Influence of pH

Total uptake of picloram by both alfalfa and barley decreased as the pH of the treatment solution was increased. The data plotted in Figure 1 show that an increase in pH from 3.0 to 4.5 was accompanied by a drastic reduction in the amount of picloram absorbed, whereas in the pH range 4.5 to 7.0 little or no further reduction was recorded in the amount absorbed by alfalfa plants and only a slight reduction was observed in the amount taken up by barley plants. Total absorption at pH 3.0 was approximately three times that at pH 4.5. The pK of the acid is reported to be either 3.6 (97) or 4.1 (45), hence the undissociated form of the molecule may be more readily absorbed than the anionic form. These results confirm earlier work that uptake of picloram and other weak acid growth regulators is dependent on the pH of the external medium and support the contention that uptake is partially dependent on the availability of undissociated molecules (45, 97, 110).

Uptake over Time

Figures 2 and 3 give data on the uptake of picloram over time by whole plants, intact roots and excised roots of alfalfa and barley plants, respectively. Both species had similar absorption patterns, consisting of an initial phase of rapid uptake followed by a brief lag period and then a prolonged phase of continuing absorption. The rate of uptake by whole alfalfa plants tended to diminish as time

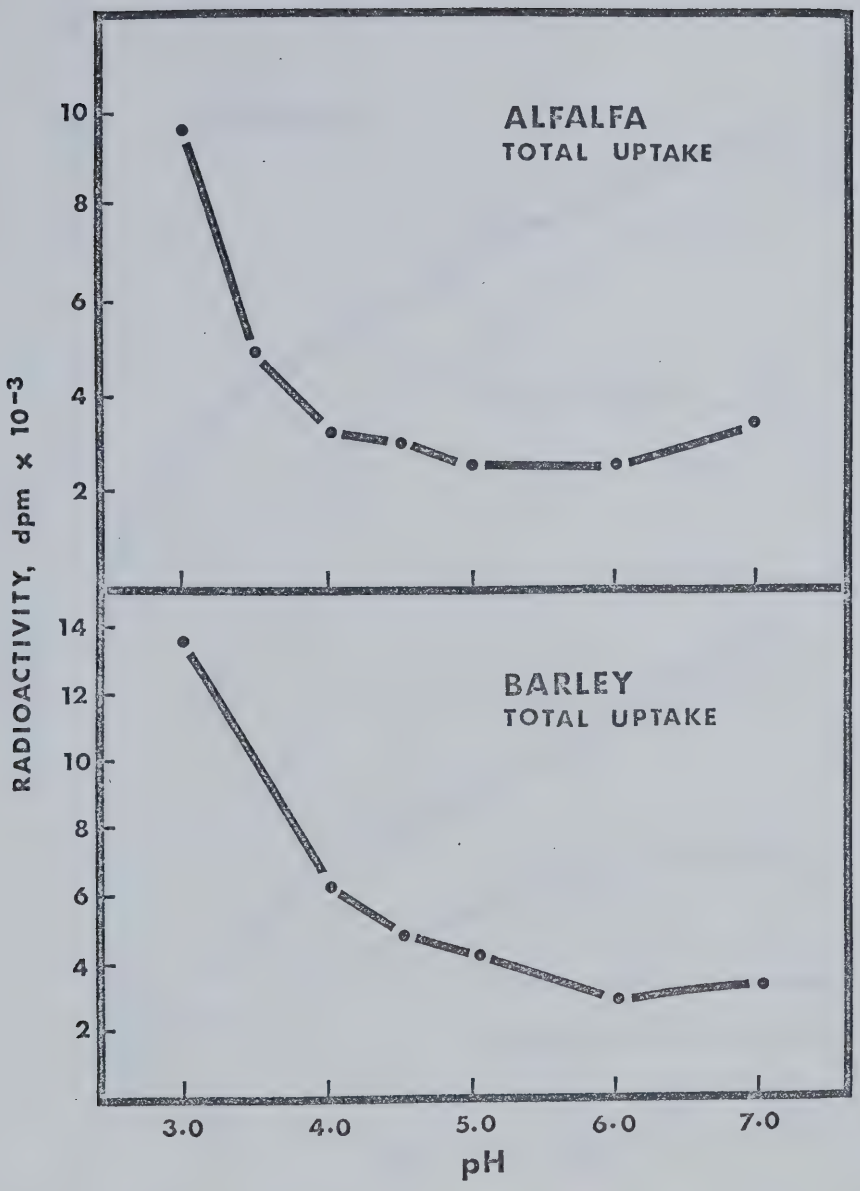


Figure 1. The influence of pH on uptake of ^{14}C-picloram by intact roots of alfalfa and barley plants. Results are expressed as dpm per plant.

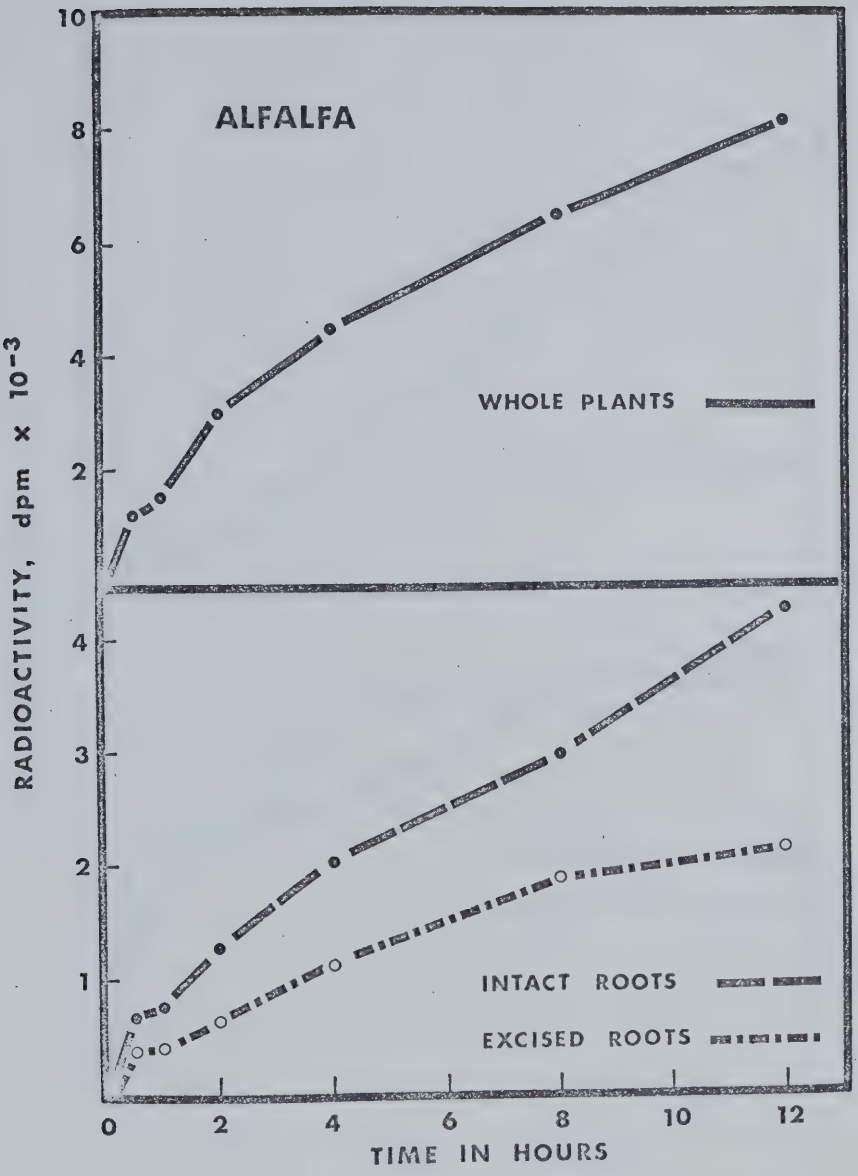


Figure 2. Time-course of uptake of ^{14}C-picloram by intact and excised roots of alfalfa plants. Results are expressed as dpm per root or plant.

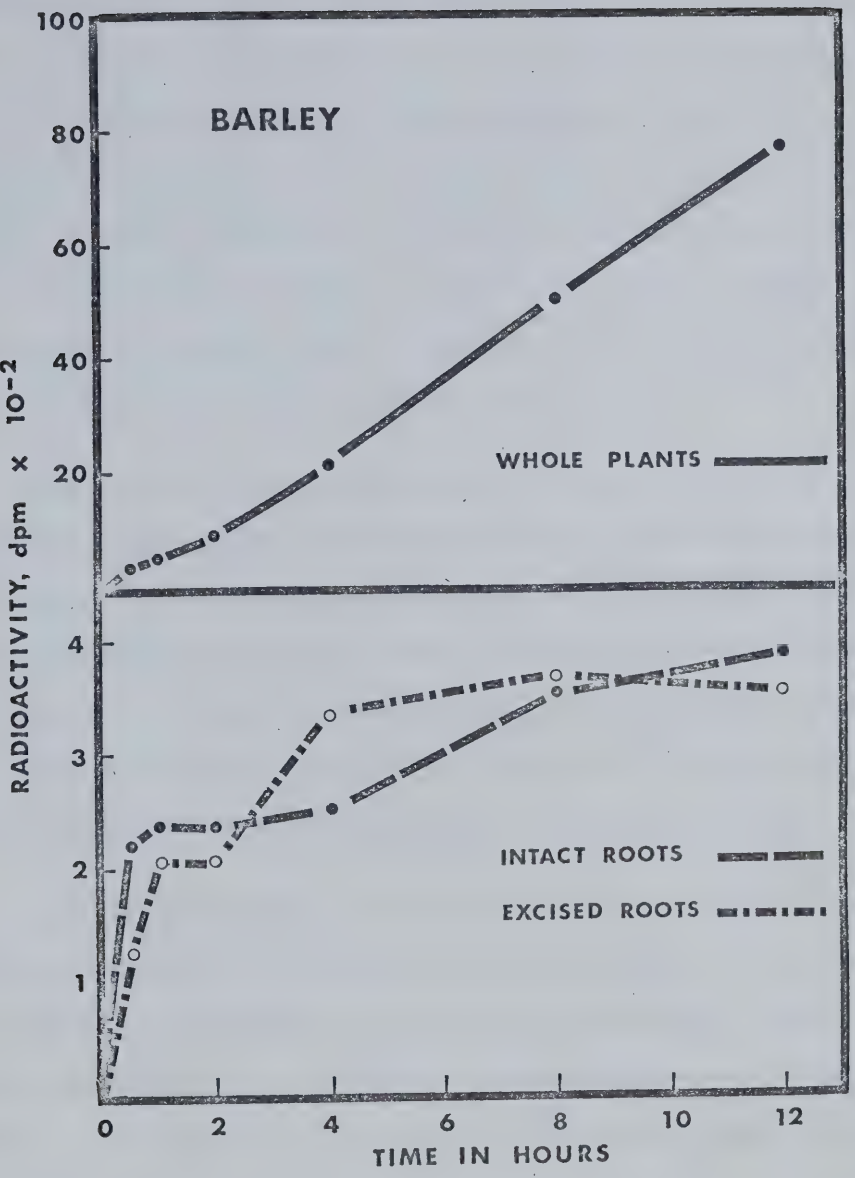


Figure 3. Time-course of uptake of ^{14}C-picloram by intact and excised roots of alfalfa plants. Results are expressed as dpm per root or plant.

progressed whereas the rate of uptake by barley plants remained essentially constant for the duration of the 12-hour treatment period. These time curves are similar to those observed by Poole and Thimann (72) for uptake of IAA by *Avena* coleoptile sections, and support the view that uptake of picloram and numerous other herbicides occurs in two separate phases: a rapid initial phase of uptake followed by a continuing phase of absorption which is generally linear with time over longer periods (45, 50, 63, 72, 79).

After 12 hr, whole alfalfa and barley plants had taken up essentially equal amounts of picloram (alfalfa, 8,125 dpm; barley, 7,733 dpm). The amount which accumulated in intact alfalfa roots (4,253 dpm), however, was more than twelve times that which accumulated in intact barley roots (354 dpm) of nearly equivalent mass. Quite obviously, the amount of picloram in alfalfa roots accounted for a greater proportion of total uptake than that in barley roots.

Accumulation in both intact and excised alfalfa roots continued throughout the 12-hour treatment period. In barley roots, picloram content reached a maximum after 4 to 6 hr and then remained constant. In the first hour, alfalfa roots accumulated approximately one-fifth and barley roots approximately one-half of the picloram present in the roots after 12 hr.

Accumulation in excised alfalfa roots was considerably less than in intact roots whereas accumulation in excised barley roots and in roots of whole plants did not differ significantly. Accumulation of picloram in alfalfa roots, but not in barley roots, appeared to be

directly affected by the presence of shoots.

Autoradiograms showing the relative amount of uptake and the distribution of picloram absorbed by roots over time are presented in Figures 4 and 5 for alfalfa and barley plants, respectively. The autoradiographic results are consistent with the data presented in Figures 2 and 3. Initial uptake of picloram by roots of alfalfa and barley plants was very rapid. Within 1 hr picloram was apparent throughout the root system of both species. Quantitatively, the amount taken up by alfalfa roots was, however, considerably more than the amount absorbed by barley roots. In alfalfa roots, accumulation was most evident in the root tips. Scott and Morris (84) reported a similar accumulation of labelled picloram in root tips of pea seedlings treated in culture solution. Autoradiograms of barley roots, on the other hand, showed a more general distribution of picloram.

After 4 hr, an appreciable amount of picloram was transported to alfalfa shoots, concentrating primarily in the rapidly developing leaves and shoot apex. As time progressed, picloram continued to be absorbed and, at the end of 12 hr, it was uniformly distributed throughout the plants. In barley plants transport to the shoots was first apparent after 2 hr. As in alfalfa plants, picloram continued to accumulate in the shoots, primarily in the newer leaves. Although absorption continued for the duration of the experiment, little more picloram accumulated in the roots than what was present after the first hour.

Retention in Roots

To determine the amount of picloram retained in roots of intact

Figure 4. Autoradiograms showing the distribution of ^{14}C-picloram in alfalfa plants treated for 1/2, 1, 2, 4, 8, and 12 hr in 100 ml of nutrient solution containing 1.0 µc of ^{14}C-picloram. Plants were exposed to X-ray film for 3 weeks.

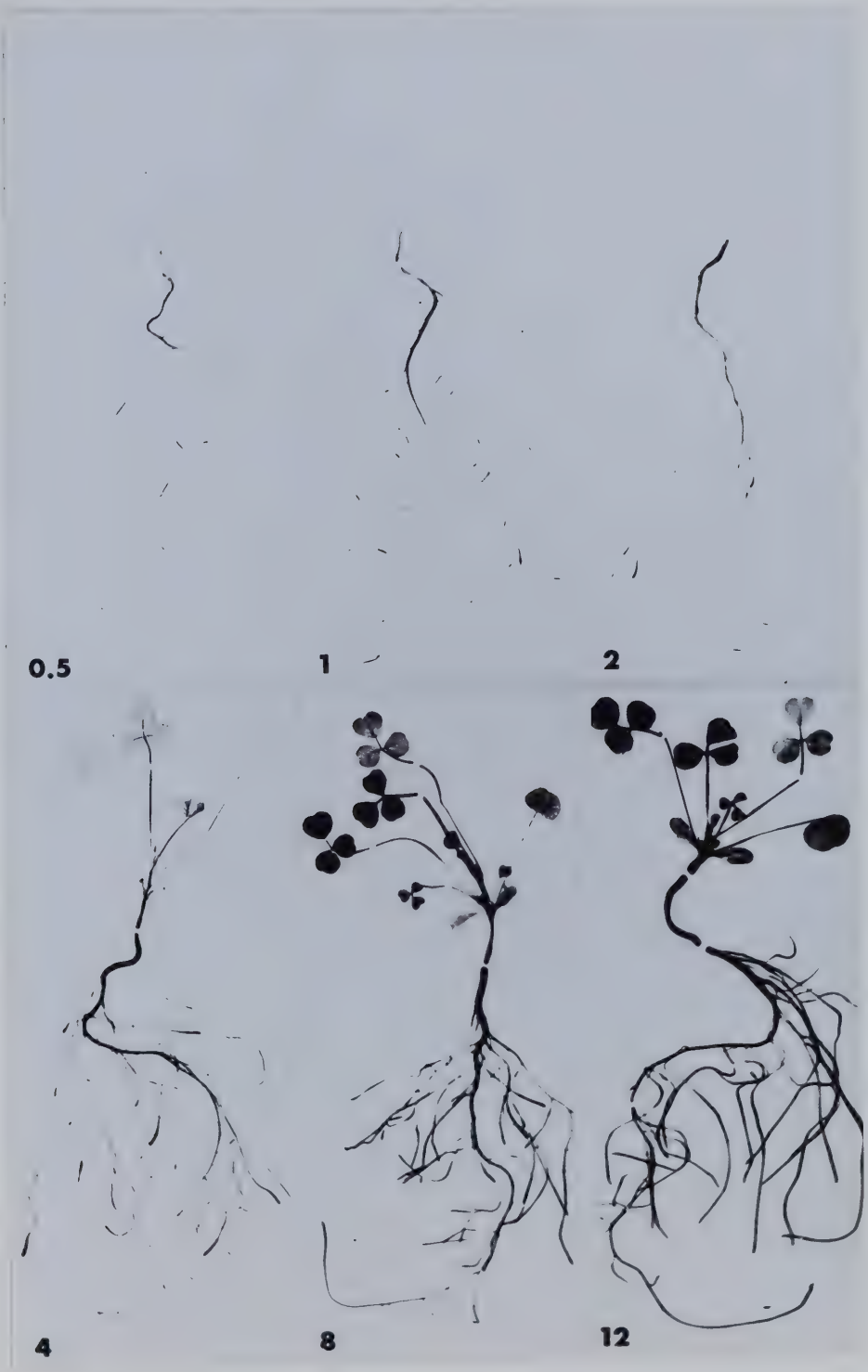
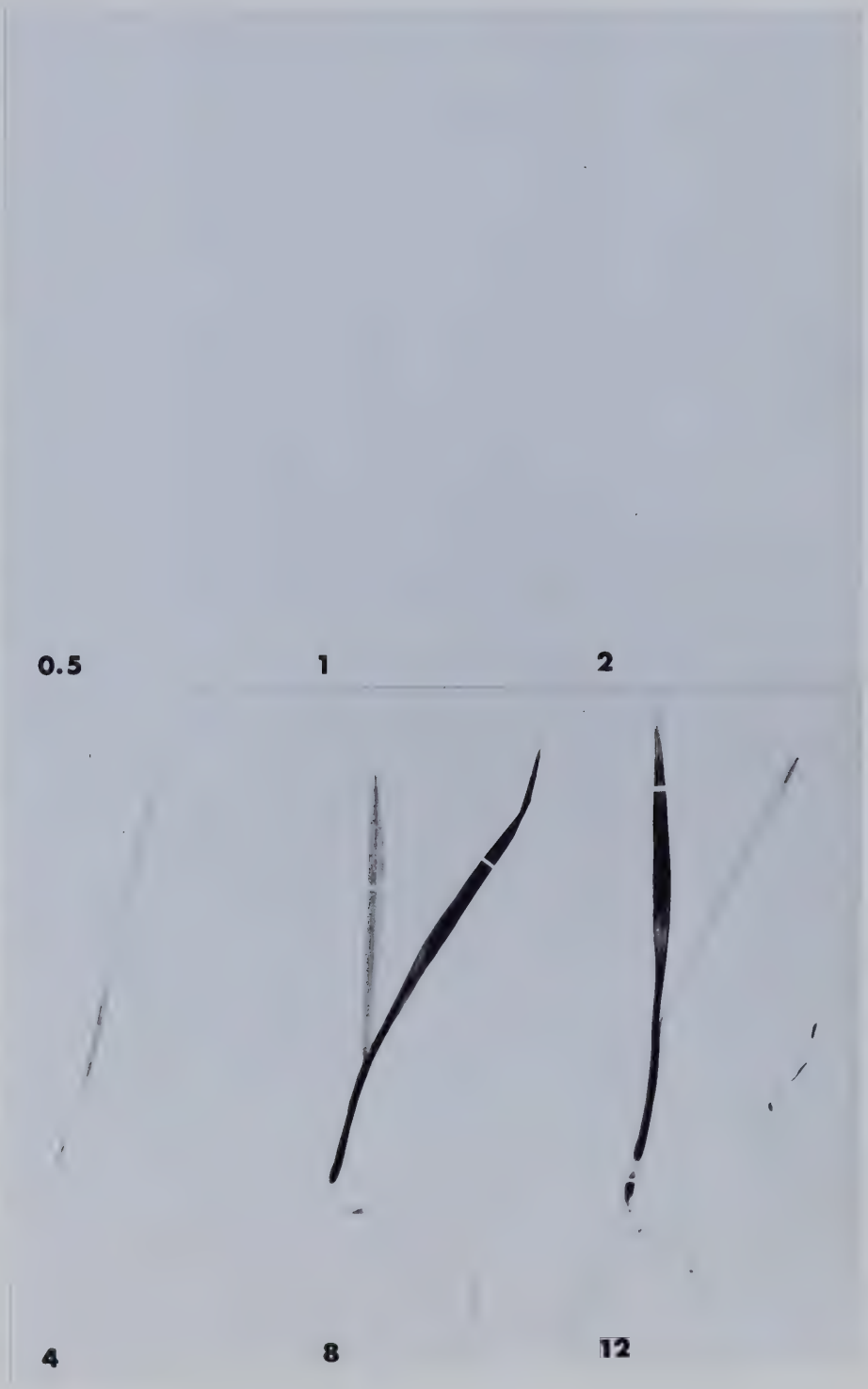

0.5
1
2
4
8
12

Figure 5. Autoradiograms showing the distribution of ^{14}C-picloram in barley plants treated for 1/2, 1, 2, 4, 8, and 12 hr in 100 ml of nutrient solution containing 1.0 µc of ^{14}C-picloram. Plants were exposed to X-ray film for 3 weeks.


0.5
1
2
4
8
12

plants over time, plants were first placed in ^{14}C-picloram treatment solution for 4 hr and then transferred to unlabelled nutrient solution for 1, 2, 4, 8, 12, and 24 hr. Figure 6 shows that picloram was rapidly transported from the roots to the shoots and/or lost from the roots to the external solution. Only 25% of that which accumulated in alfalfa roots in the 4-hour treatment period was retained after transfer to the unlabelled solution for 4 hr. The amount retained after 24 hr was just 15% of that initially present in the roots. These results are in close agreement with those of Scott and Morris (84) who reported that only 15% of the picloram initially present in roots of pea seedlings treated under conditions similar to those of the present study remained after transfer to unlabelled medium for 24 hr. The amount retained in barley roots after 2 hr in the unlabelled solution was approximately 40% of that which initially accumulated in the 4-hour treatment period. No further reduction in the amount retained by barley roots was observed over the remainder of the 24-hour incubation in unlabelled solution. The picloram retained 24 hr after treatment was evidently tenaciously bound in the roots.

Autoradiograms in Figure 7 and 8 of alfalfa and barley plants, respectively, agree with the results presented in Figure 6, and confirm that picloram is rapidly lost from the roots of both species upon transfer from treatment solution to unlabelled nutrient solution. Within 1 hr all of the picloram initially present in barley roots and much of what was apparent in alfalfa roots was no longer evident. Substantial amounts of picloram were, however, retained in alfalfa root tips. No picloram was detected in roots of either species at the end of 8 hr.

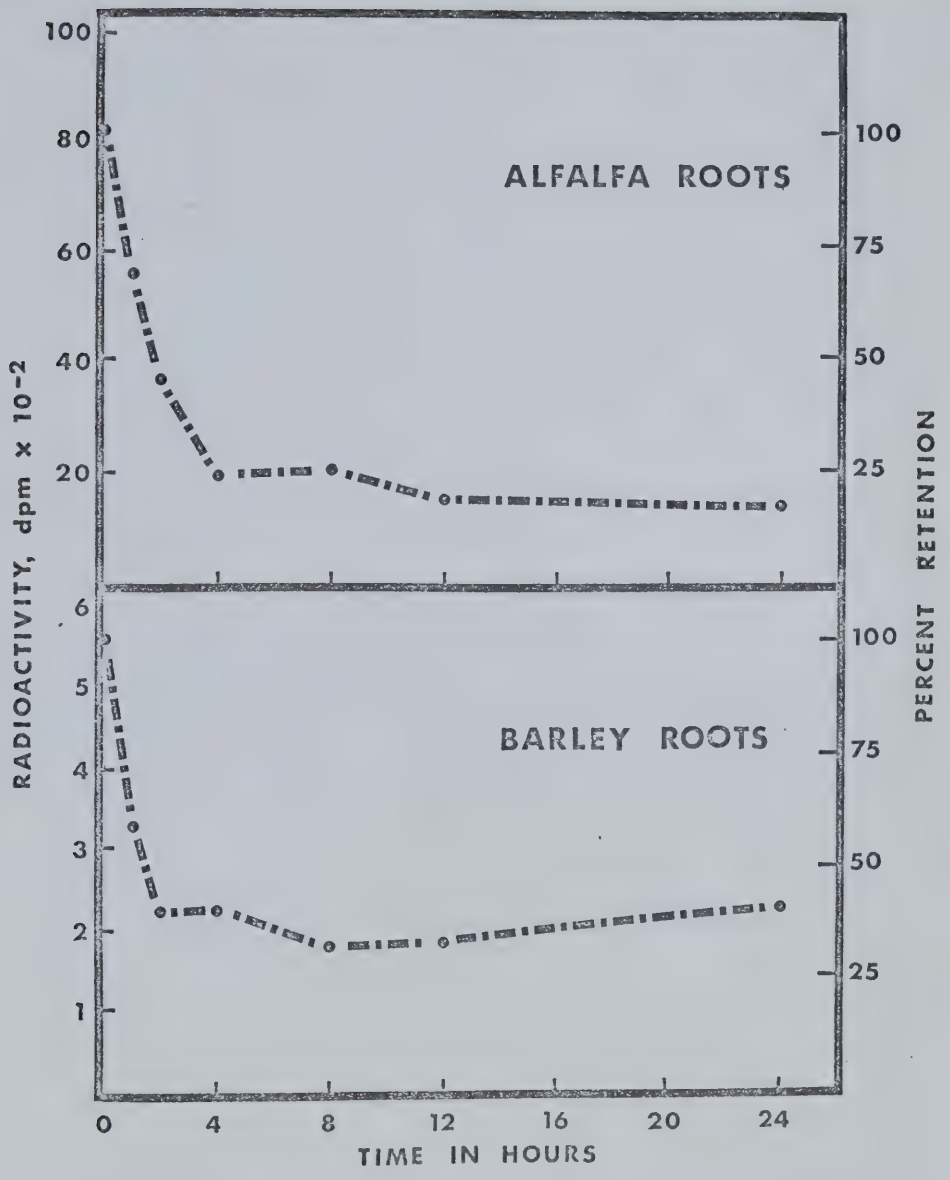


Figure 6. Time-course of the retention of ^{14}C-picloram in roots of treated alfalfa and barley plants. Plants were incubated in 30 ml of unlabelled nutrient solution following treatment for 4 hr in 100 ml of nutrient solution containing 1.0 µc of ^{14}C-picloram. Results are expressed as dpm per root (left axis) and as percentage of the amount present immediately after treatment (right axis).

Figure 7. Autoradiograms showing the distribution of ^{14}C-picloram in alfalfa plants 0, 1, 2, 4, 8, and 12 hr following treatment for 4 hr in 100 ml of nutrient solution containing 1.0 µc of ^{14}C-picloram. Plants were exposed to X-ray film for 3 weeks.

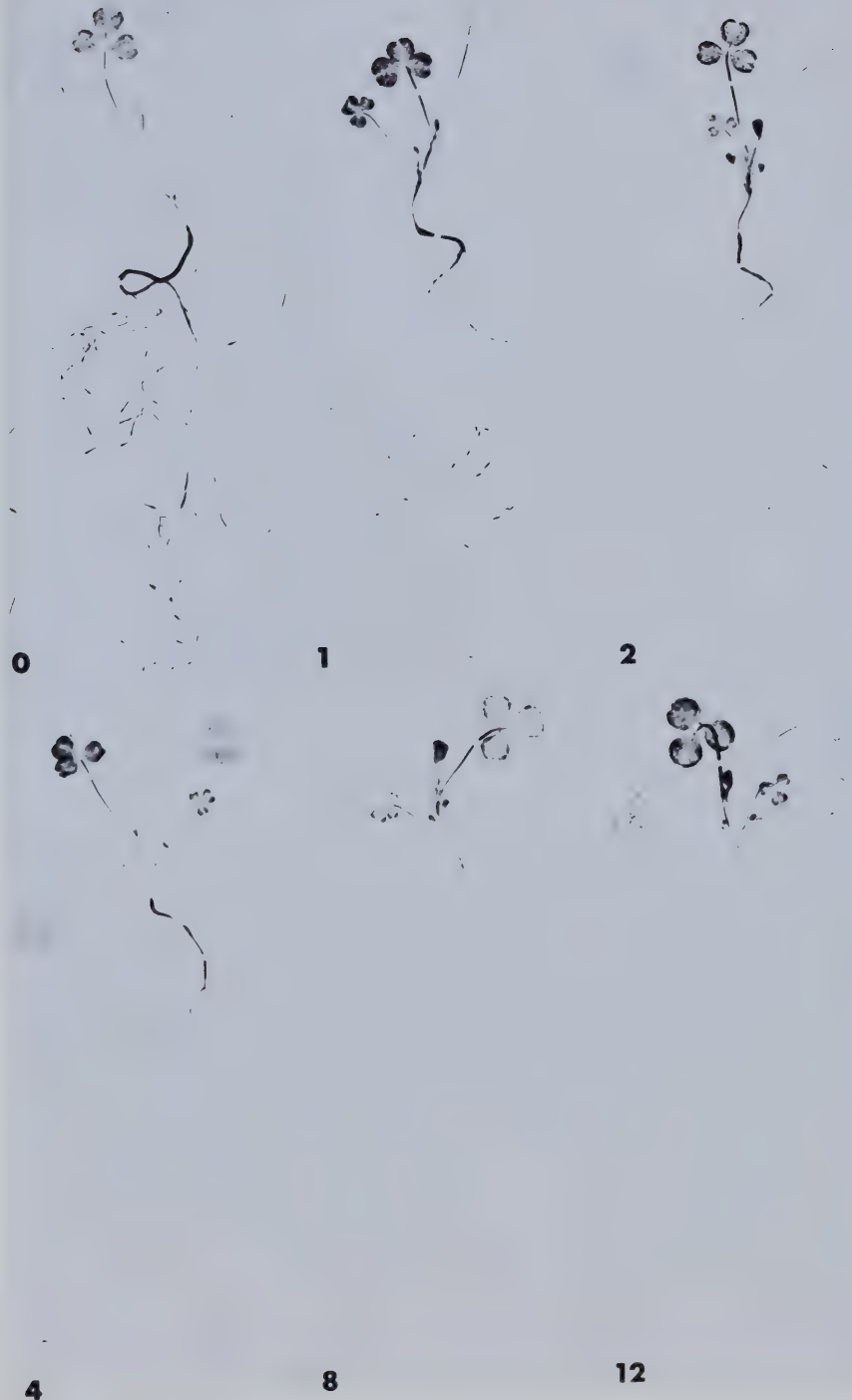
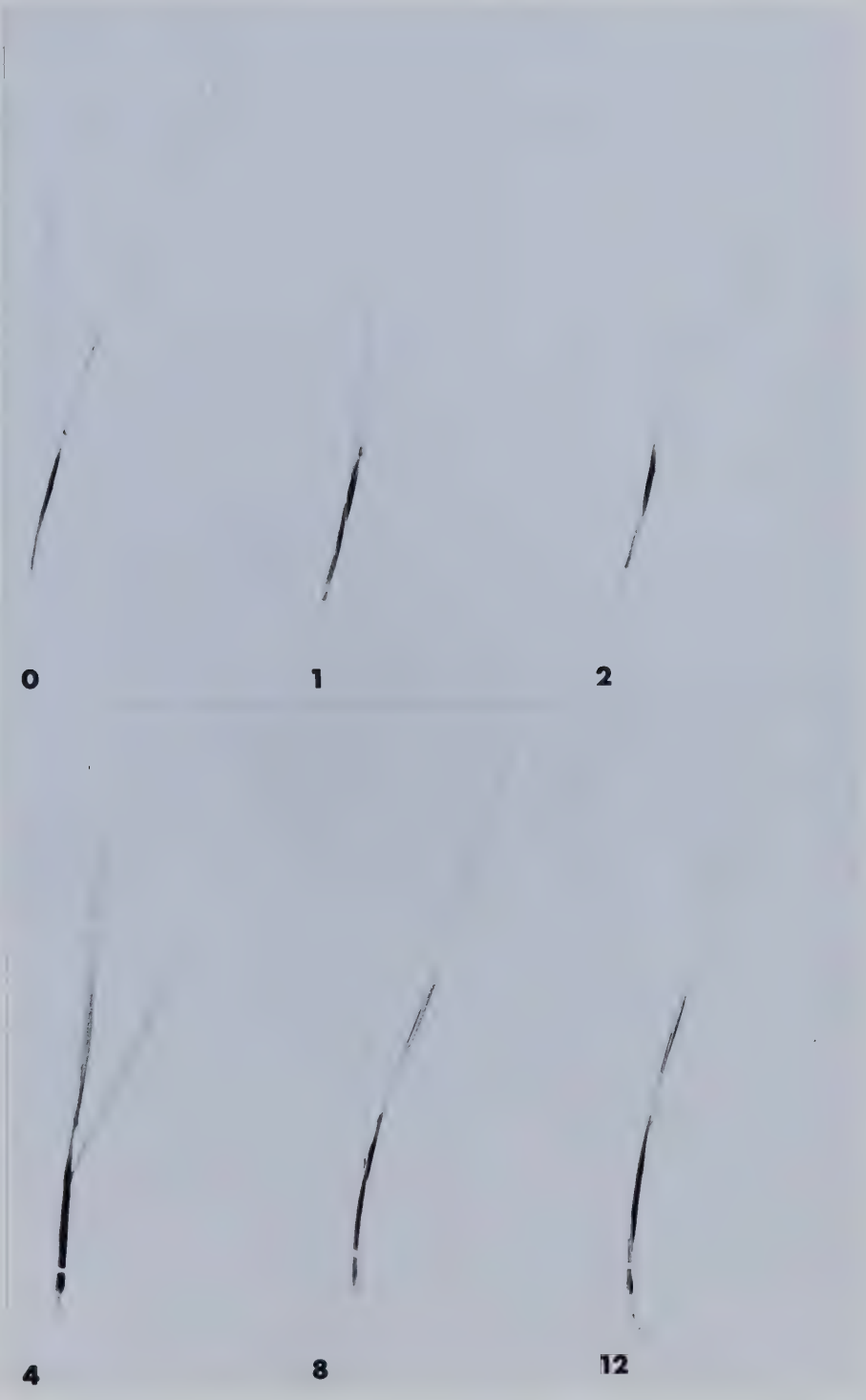

0
1
2
4
8
12

Figure 8. Autoradiograms showing the distribution of ^{14}C-picloram in barley plants 0, 1, 2, 4, 8, and 12 hr following treatment for 4 hr in 100 ml of nutrient solution containing 1.0 µc of ^{14}C-picloram. Plants were exposed to X-ray film for 3 weeks.


0
1
2
4
8
12

To ascertain whether some of the picloram taken up during the initial rapid phase of absorption is contained in the free space of the root and whether any is held in an exchangeable form within the root, plants were exposed to a 10 μM ^{14}C-picloram solution for 2 hr prior to incubation in 0, 10, 50 μM unlabelled picloram solutions for 1/4, 1/2 and 1 hr.

The data presented in Table 1 show that intact roots of alfalfa and barley plants lost about one-half of the picloram which accumulated in the 2-hour exposure to treatment solution after only 15 minutes in unlabelled nutrient solution. Loss of picloram continued for the duration of the experiment. At the end of 1 hr, only one-sixth of the amount initially present in alfalfa roots was retained whereas approximately one-quarter of the amount initially present in barley roots remained. The loss from alfalfa roots was accompanied by a reduction in the total picloram retained in whole plants, suggesting a movement of the compound out of the roots into the external solution. Even though the amount of picloram in barley roots was significantly decreased after 1 hr in unlabelled nutrient solution, total picloram in the whole plants did not appear to be reduced. Variability between replicates and the fact that the roots of barley plants initially contained only one-eighth of the total picloram in a plant could have masked an actual loss to the external solution.

The fraction of picloram retained in roots after 1 hr was considerably less in these experiments than in the results reported in Figure 6. Since the uptake period was only half as long, less picloram was present in the roots upon transfer to the unlabelled solutions.

TABLE 1

Loss of ^{14}C-picloram from intact roots of alfalfa and barley plants incubated for 1/4, 1/2 and 1 hr in nutrient solution containing unlabelled picloram (0, 10, 50 µM)[a]

Time hr	unlabelled picloram concentration					
	0 µM		10 µM		50 µM	
	root dpm[b]	total dpm	root dpm	total dpm	root dpm	total dpm
Alfalfa						
0	4,248 a	6,954	4,248 a	6,954	4,248 a	6,954
1/4	2,261 b	4,450	1,597 bc	3,474	1,554 bc	3,670
1/2	1,480 cd	4,992	1,228 cde	4,380	1,296 cde	4,496
1	686 e	3,282	810 de	3,500	1,013 cde	3,986
Barley						
0	245 a	1,500	245 a	1,500	245 a	1,500
1/4	132 ab	1,532	139 ab	1,562	136 ab	1,591
1/2	127 ab	1,507	122 ab	1,313	104 b	1,685
1	69 b	1,667	58 b	1,234	47 b	1,296

[a]Plants were exposed to 10 ml of treatment solution for 2 hr prior to incubation in 100 ml of unlabelled solution.

[b]Results are expressed as dpm per root or plant. Data presented are means of 3 replicates. Means followed by the same letter are not significantly different from one another at the 5% level of probability as determined by Duncan's new multiple range test.

Presumably, over a longer treatment period a greater proportion of the picloram absorbed is irreversibly taken up by the root and cannot be released.

The loss of picloram to unlabelled picloram solution was not significantly greater than loss to nutrient solution. A tendency toward a somewhat greater loss of picloram to solutions containing unlabelled picloram was nevertheless apparent, particularly from barley roots. Although a large proportion of the picloram initially present in the root appeared to be able to freely diffuse out of the free space, a small amount may have been adsorbed in an exchangeable form within the root.

The Effect of External Picloram Concentration

If uptake mechanisms are under metabolic control or if adsorption is a principal means of uptake, saturation kinetics would be expected to hold over a wide range of external picloram concentration. If, on the other hand, absorption occurs by passive processes such as diffusion or mass flow, a direct proportionality would exist.

The data presented in Table 2 show that total picloram uptake from solutions ranging from 0.5 to 20 μM was directly proportional to the external concentration over 1/2 and 4-hour treatment periods.

A linear relationship between total uptake and picloram concentration in the treatment solution over a 1/2-hour period may indicate that passive processes are involved in the initial phase of absorption. Indeed, Isensee *et al.* (45) observed that picloram absorption by oat

TABLE 2

The effect of external concentration on uptake of ^{14}C-picloram by intact roots of alfalfa and barley plants during 1/2 and 4-hr treatment periods

Picloram conc., µM	1/2 hr		4 hr	
	Root dpm[a]	Total dpm	Root dpm	Total dpm
Alfalfa				
0.5	45	57	240	586
1.0	110	134	716	1,489
5.0	523	603	2,572	6,188
10.0	1,104	1,241	4,473	10,952
20.0	1,632	1,981	7,684	20,630
Barley				
0.5	14	24	56	255
1.0	21	33	49	268
5.0	143	191	153	1,104
10.0	250	338	782	2,741
20.0	638	914	1,370	6,155

[a]Results are expressed as dpm per root or plant. Data presented are means of 3 replicates.

and soybean roots treated for 30 or 40 minutes was directly related to picloram concentration in the treatment solution, and concluded that initial absorption was a passive process governed by concentration and diffusion equilibrium. Similar results have been reported for the initial entry of other herbicides and growth regulators considered to be initially absorbed by passive mechanisms (22, 14, 106). Lack of saturation kinetics over a 4-hour treatment period does not, however, preclude the possibility that active processes are involved in the prolonged phase of continuing picloram absorption.

The Effect of Metabolic Inhibitors

If metabolic energy is required to drive 'active' uptake mechanisms, picloram absorption by roots would be expected to decrease in the presence of respiratory inhibitors. To determine the effect of the uncoupling agent, DNP, on uptake of picloram by roots of alfalfa and barley, intact plants were placed for 4 hr in ^{14}C-picloram solutions containing various concentrations of DNP.

The results in Figure 9 indicate that 1 mM DNP markedly inhibited uptake by both species. In 4 hr, total uptake of picloram by alfalfa and barley plants was only 15% and 25%, respectively, of that taken up by plants treated in solution containing no inhibitor.

Uptake by alfalfa plants was enhanced at 0.01 mM DNP, whereas uptake by barley plants was slightly inhibited. At low concentration DNP has been shown to stimulate respiration and simultaneously increase solute absorption (14, 40, 45, 93). At inhibitory concentrations, DNP blocks the synthesis of all high energy compounds and brings about the

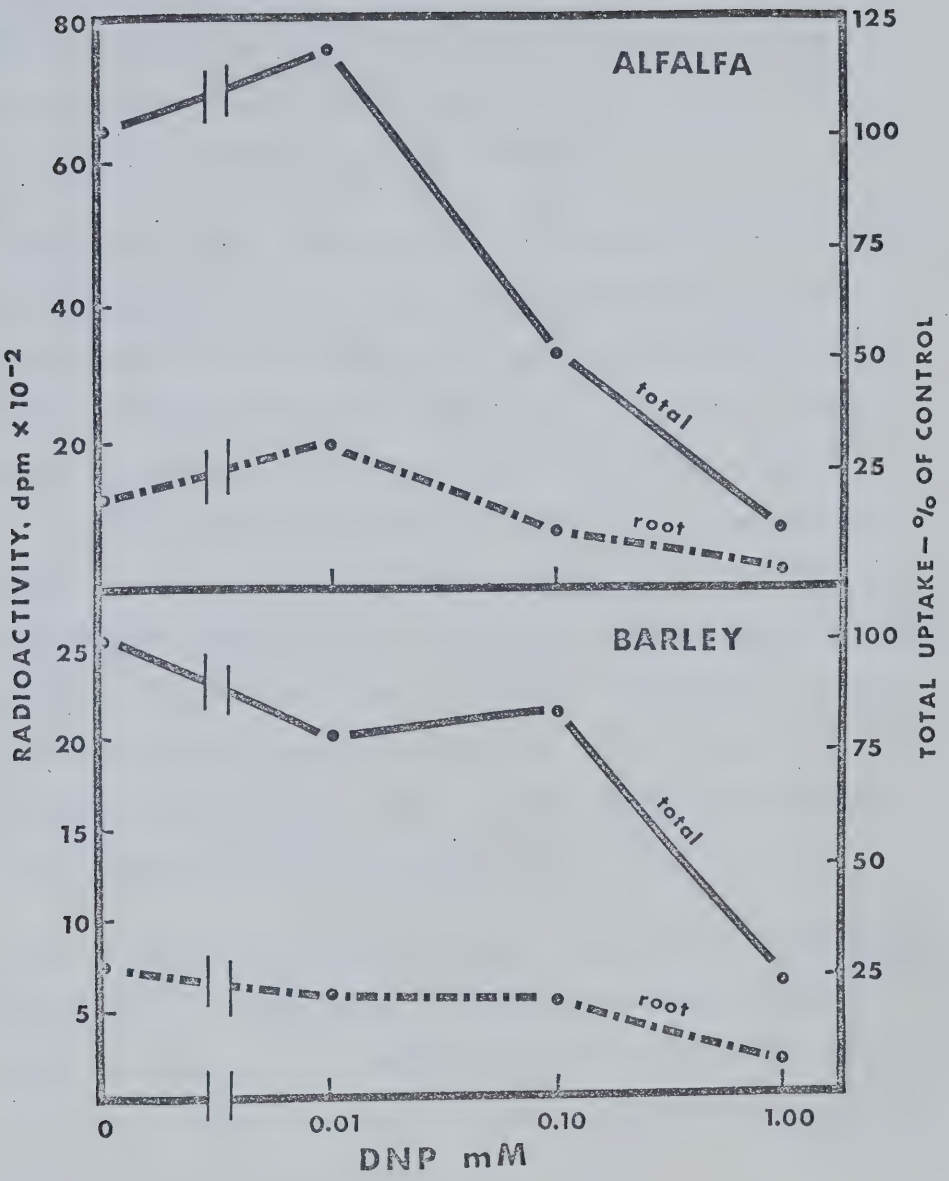


Figure 9. The effect of various concentrations of DNP on uptake of ^{14}C-picloram by intact roots of alfalfa and barley plants. Results are presented as dpm per root or plant (left axis) and as percentage of total uptake by control plants (right axis).

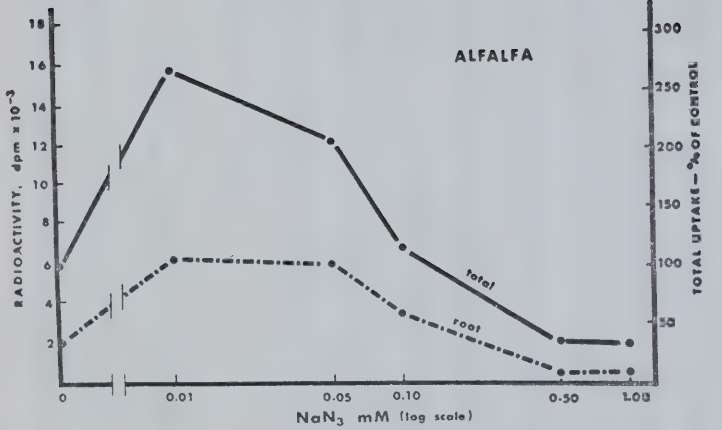
hydrolysis of any ATP molecules which may have accumulated prior to treatment. Christie and Leopold (14) observed that 0.001 mM DNP increased absorption of IAA by corn coleoptiles whereas higher concentrations markedly inhibited entry of the auxin.

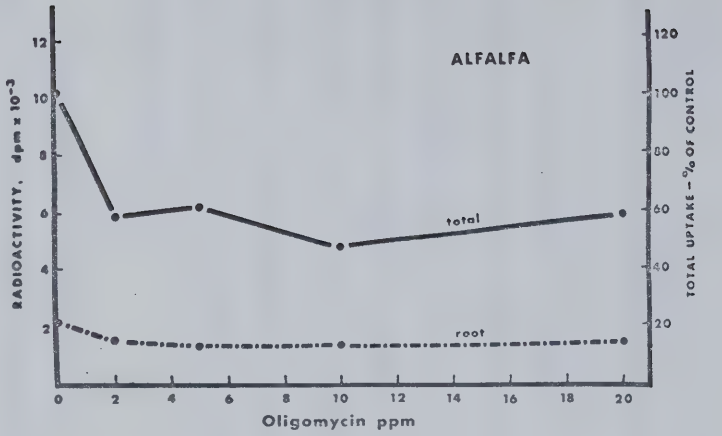
Sodium azide (NaN_3) and oligomycin are considered to be inhibitors of oxidative phosphorylation. Neither of the compounds acts as an uncoupler and they do not, therefore, promote the hydrolysis of ATP and other high energy compounds. Sodium azide is generally thought to inhibit electron transfer along the cytochrome chain as well as inhibit reactions involved in the production of ATP. Oligomycin is considered to block one of the terminal steps of the phosphorylation sequence, thereby inhibiting the conversion of high energy phosphorylated intermediates to ATP. The effect of various concentrations of both NaN_3 and oligomycin on uptake of picloram by roots of alfalfa and barley is illustrated in Figure 10. The experiments were conducted in a manner similar to that previously described for DNP.

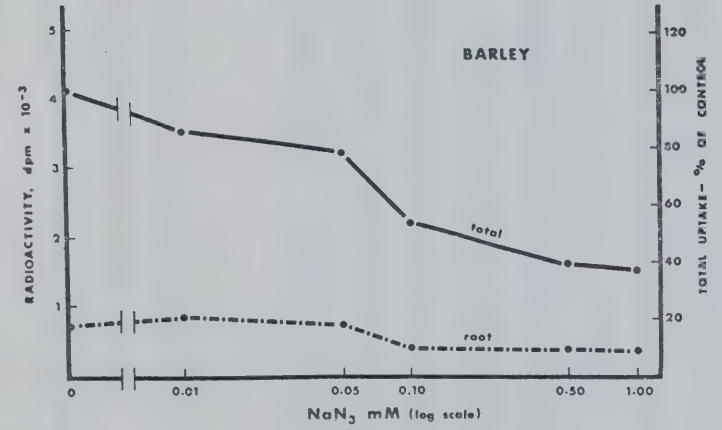
High concentrations of NaN_3 markedly decreased picloram absorption by both alfalfa and barley plants. Oligomycin inhibited picloram absorption by both species at concentrations as low as 2 ppm. Further increasing the concentration did not result in a greater reduction in the amount of picloram absorbed.

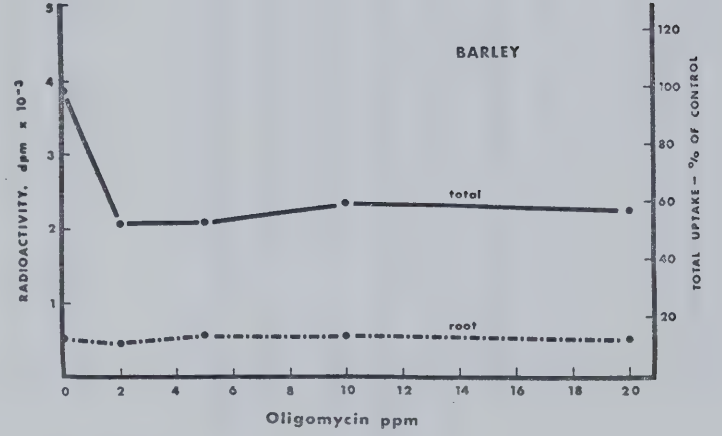
Sodium azide, like DNP, stimulated uptake at low concentrations. This increased absorption may again be attributed to increased respiratory activity induced by low concentrations of NaN_3. Low concentrations of NaN_3 have repeatedly been shown to stimulate the

Figure 10. The effect of various concentrations of NaN_3 and oligomycin on uptake of ^{14}C-picloram by intact roots of alfalfa and barley plants. Results are expressed as dpm per root or plant (left axes) and as percentage of total uptake by control plants (right axes).


ALFALFA
RADIOACTIVITY, dpm x 10^{-3}
TOTAL UPTAKE — % OF CONTROL
total
root
0
0.01
0.05
0.10
0.50
1.00
NaN_3 mM (log scale)


ALFALFA
RADIOACTIVITY, dpm x 10^{-3}
TOTAL UPTAKE — % OF CONTROL
total
root
Oligomycin ppm


BARLEY
RADIOACTIVITY, dpm x 10^{-3}
TOTAL UPTAKE — % OF CONTROL
total
root
0
0.01
0.05
0.10
0.50
1.00
NaN_3 mM (log scale)


BARLEY
RADIOACTIVITY, dpm x 10^{-3}
TOTAL UPTAKE — % OF CONTROL
total
root
Oligomycin ppm

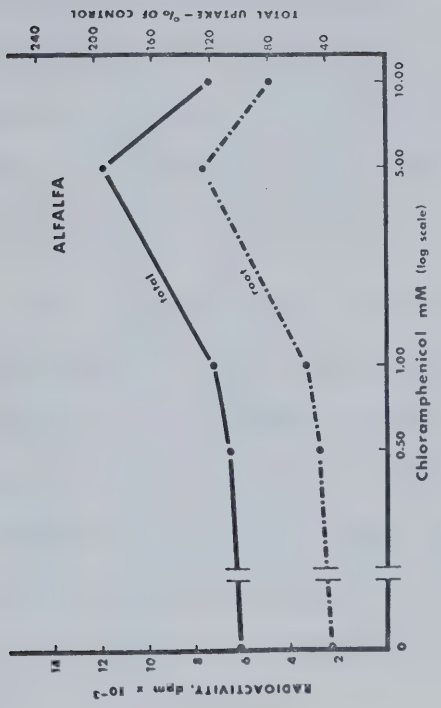
respiration of plant tissues, while high concentrations have been shown to depress respiratory metabolism (1, 73).

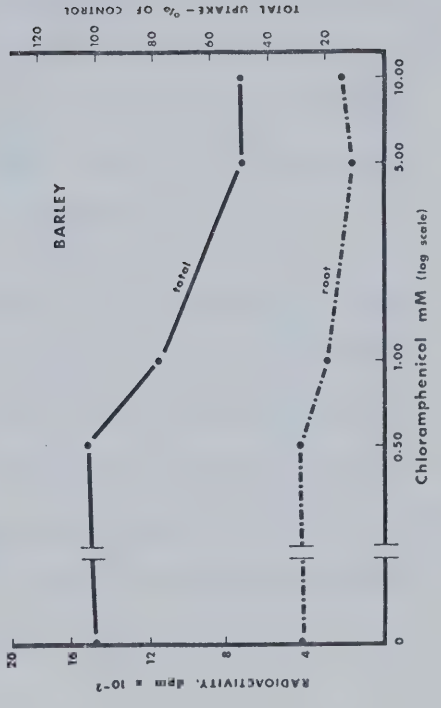
Studies similar to those involving respiratory inhibitors were conducted with two inhibitors of protein synthesis, cycloheximide and chloramphenicol. The results (Figure 11) show that total picloram absorption by alfalfa plants was markedly reduced by all concentrations of cycloheximide, whereas total absorption by barley was relatively unaffected. Chloramphenicol, on the other hand, actually enhanced total absorption by alfalfa but inhibited uptake by barley at higher concentrations.

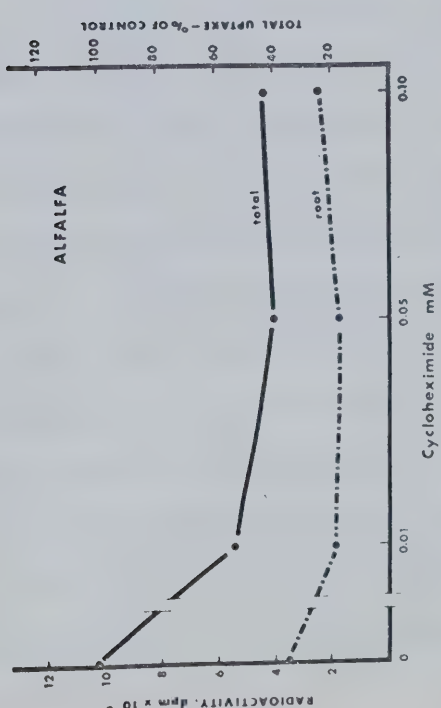
Despite the fact that both cycloheximide and chloramphenicol have traditionally been considered specific inhibitors of protein synthesis, the inhibitory effect of cycloheximide on uptake of picloram by alfalfa and of chloramphenicol on uptake by barley is not necessarily attributable to an inhibition of protein synthesis. Indeed, Ellis and MacDonald (25) have recently reported that the inhibitory effect of both compounds on active ion uptake by plant tissues is mediated via interference with energy transfer and oxidative phosphorylation processes. The increase in total uptake of picloram by alfalfa plants treated in a solution containing chloramphenicol may be analagous to similar increases observed in plants treated with low concentrations of DNP and NaN_3.

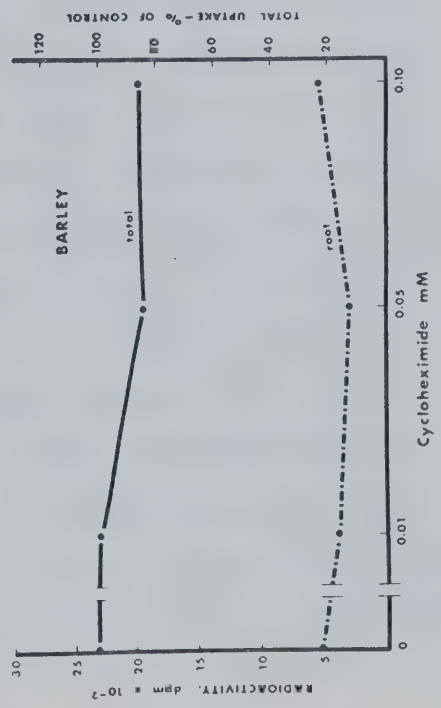
From the results of the experiments previously described it was apparent that a decrease in total picloram absorption was, in all cases, accompanied by a proportional decrease in the relative amount

Figure 11. The effect of various concentrations of cycloheximide and chloramphenicol on uptake of ^{14}C-picloram by intact roots of alfalfa and barley plants. Results are expressed as dpm per root or plant (left axes) and as percentage of total uptake by control plants (right axes).


ALFALFA
total
root
RADIOACTIVITY, dpm x 10⁻³
TOTAL UPTAKE – % OF CONTROL
Chloramphenicol mM (log scale)


BARLEY
total
root
RADIOACTIVITY, dpm x 10⁻²
TOTAL UPTAKE – % OF CONTROL
Chloramphenicol mM (log scale)


ALFALFA
total
root
RADIOACTIVITY, dpm x 10⁻³
TOTAL UPTAKE – % OF CONTROL
Cycloheximide mM


BARLEY
total
root
RADIOACTIVITY, dpm x 10⁻²
TOTAL UPTAKE – % OF CONTROL
Cycloheximide mM

of picloram present in the roots. To substantiate that the effect of the inhibitors was actually on the absorptive mechanisms in the roots and not on transport processes which could be affected by the shoots, both intact and excised roots were treated for 4 hr in solutions containing inhibitory concentrations of the compounds.

The graphs in Figure 12 show that all of the inhibitors except chloramphenicol reduced uptake of picloram by both intact plants and excised roots of alfalfa. Similarly, all of the inhibitors except cycloheximide substantially reduced uptake by both intact plants and excised roots of barley. These results concur with the results of the previous studies and suggest that the effect of metabolic inhibitors is, at least in part, on absorptive mechanisms inherently operative in roots.

Figures 13 and 14 show the effect of NaN_3 on uptake of picloram over time by alfalfa and barley plants, respectively. Initial uptake of picloram by alfalfa plants was little affected by the inhibitor. At the end of one hour, there was no significant difference between the amounts absorbed from treatment solution with or without NaN_3. Continuing absorption was greatly affected, however. In the presence of NaN_3 little further total uptake occurred, whereas without the inhibitor uptake continued for an extended period. At the end of the treatment period, uptake in the presence of inhibitor was only one-seventh of that in control plants. Similar trends were evident in the amounts accumulated in roots. Uptake was little affected during the first hour of absorption whereas prolonged absorption was markedly reduced.

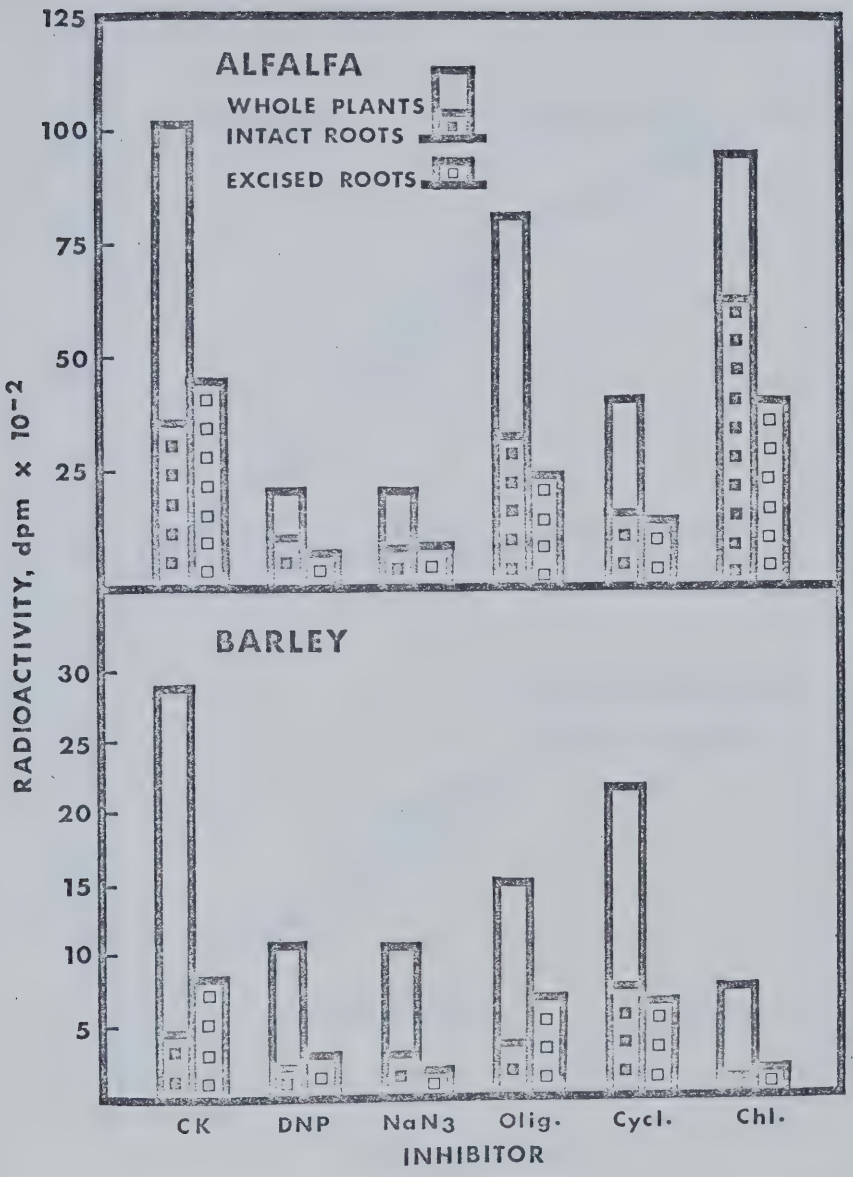


Figure 12. The effect of DNP (0.1 mM), NaN_3 (0.5 mM), oligomycin (2 ppm), cycloheximide (0.05 mM), and chloramphenicol (0.05 mM) on uptake of ^{14}C-picloram by intact and excised roots of alfalfa and barley plants. Results are expressed as dpm per root or plant.

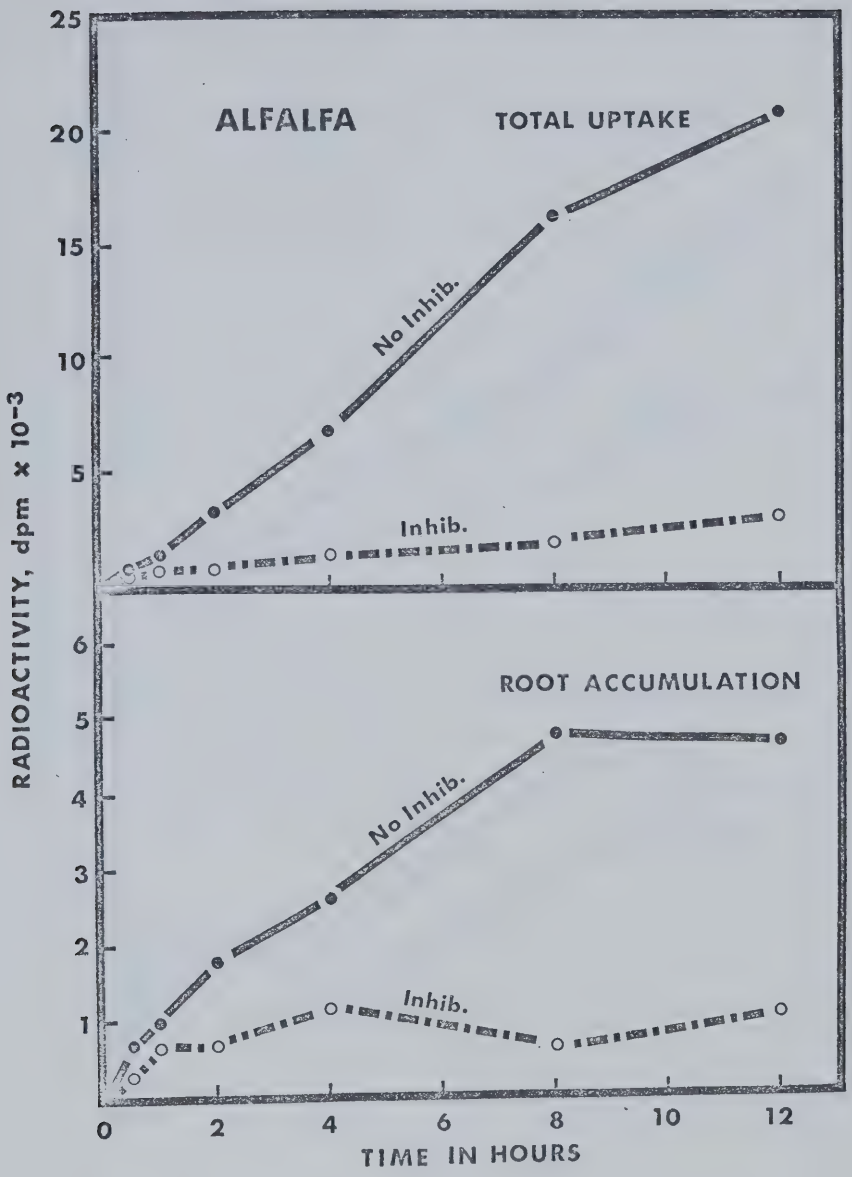


Figure 13. The effect of NaN_3 (1.0 mM) on uptake of ^{14}C-picloram over time by intact roots of alfalfa plants. Results are presented as dpm per root or plant.

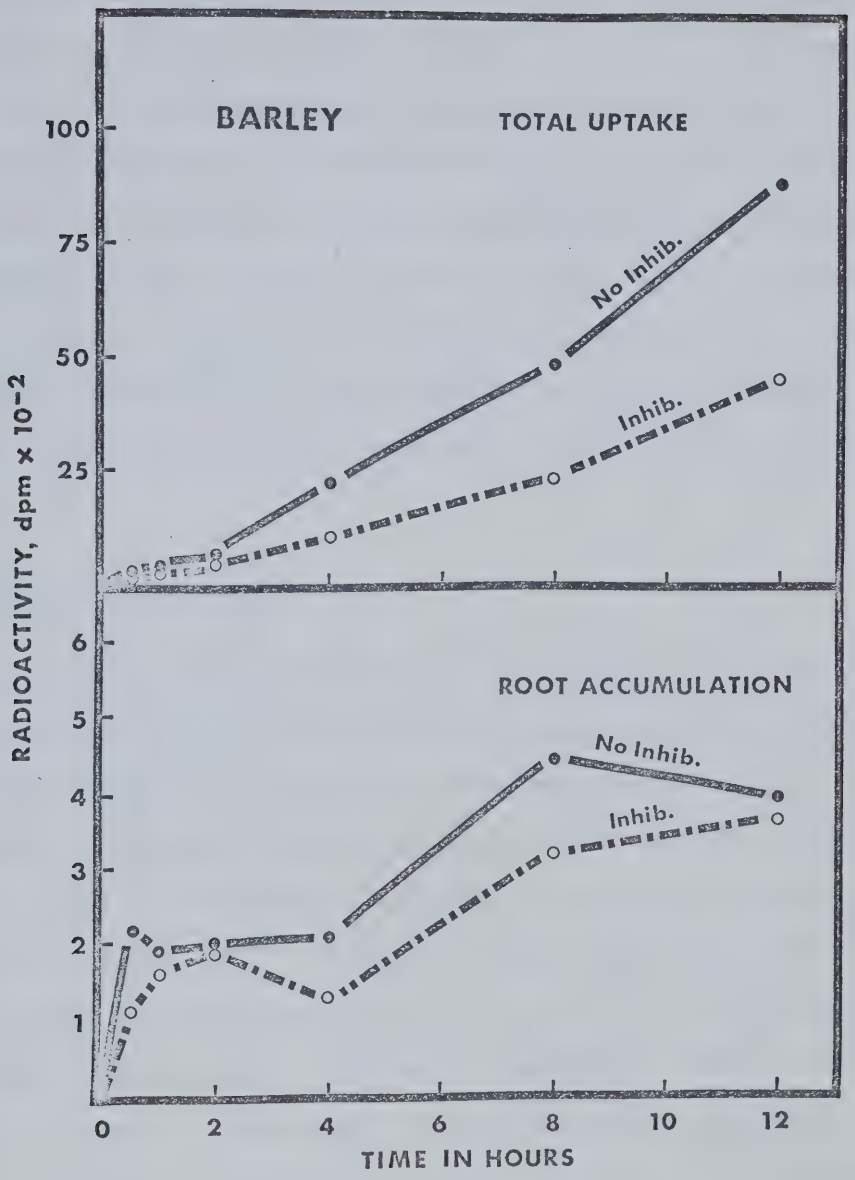


Figure 14. The effect of NaN_3 (1.0 mM) on uptake of ^{14}C-picloram over time by intact roots of barley plants. Results are presented as dpm per root or plant.

Initial accumulation in barley roots was not affected by NaN_3. Continuing accumulation was greatly reduced, however, such that at the end of the 12-hour treatment period only about one-half as much picloram was absorbed in the presence of NaN_3. The amount of picloram accumulated in the roots of barley was not significantly affected by NaN_3 although it was somewhat less than in control plants throughout the experiment. Initial entry of picloram into roots of both alfalfa and barley apparently was controlled by passive processes whereas continuing absorption seemingly involved the expenditure of metabolic energy.

The Effect of Temperature

The data recorded in Table 3 show that throughout the duration of the experiments alfalfa and barley plants accumulated more picloram when roots were treated at 23°C than when roots were treated at 13°C. Temperature coefficients (Q_{10} values) for the first hour of uptake by alfalfa plants were less than 1.4 whereas for subsequent absorption they were greater than 2.0. Temperature coefficients for uptake by barley plants were approximately 1.0 for the first 2 hr of absorption, after which they increased progressively, reaching a value of 6.6 between 8 and 12 hr. These results suggest that uptake during the initial stage of rapid absorption occurs predominantly by physical mechanisms and that uptake during the prolonged phase of continuing absorption is mediated by metabolically controlled processes.

The effect of root temperature on translocation was negligible. The ratios of the activity in the shoots to that in the roots (S/R),

TABLE 3

The effect of root temperature on uptake and translocation of ^{14}C-picloram by intact roots of alfalfa and barley plants over time[a]

Time hr	13°			23°			Q_{10}
	Root dpm[b]	Total dpm	S/R[c]	Root dpm	Total dpm	S/R	Total
Alfalfa							
0							
1/2	572	699	0.22	799	984	0.23	1.4
1	1,066	1,400	0.31	1,426	2,004	0.40	1.4
2	1,304	2,160	0.65	2,241	3,697	0.94	2.0
4	2,111	4,753	1.25	2,865	8,803	2.07	2.2
8	2,410	8,623	2.57	5,089	19,816	2.89	2.8
12	3,119	15,530	3.97	8,947	39,294	3.39	2.8
Barley							
0							
1/2	33	56	0.69	48	69	0.47	1.2
1	47	101	1.15	87	116	0.33	1.0
2	79	170	1.15	131	187	0.43	1.0
4	184	361	0.96	202	584	1.89	2.5
8	115	506	5.18	156	1,337	7.57	5.2
12	41	638	14.56	141	2,214	14.70	6.6

[a]Roots were treated *en masse* in 100 ml of treatment solution at 13° or 23° C (water bath) while air temperature remained at 21 ± 1°C.

[b]Results are expressed as dpm per root or plant.

[c]Ratio of the activity in the shoots to that in the roots.

shown in Table 3, give an indication of the relative transport of picloram from roots to shoots. At the end of the 12-hour treatment period, alfalfa plants had S/R ratios of 3.97 and 3.39 while barley plants had ratios of 14.56 and 14.70 at 13° and 23°C, respectively. Obviously a much greater proportion of the total picloram absorbed was transported to barley shoots than to alfalfa shoots.

Accumulation of picloram in barley roots reached a maximum after 4 hr and then declined sharply, particularly in roots treated at 13°C. A number of experiments showed this trend and, although the amount accumulated at 8 and 12 hr was not always significantly less than that after 4 hr, the tendency for barley roots to retain less picloram over longer time periods appeared consistently. Since the total amount present in the plants continued to increase this reduction could not be attributed to egress from the roots but it could possibly have been due to a stimulation in the rate of transport to the shoots.

The Effect of Succinate, Sucrose and ATP

Assuming that absorption of picloram by roots is accomplished, at least in part, by active energy requiring processes, the presence of oxidizable substrates in the culture solution would be expected to enhance uptake. The results shown in Table 4 illustrate that sucrose stimulated uptake mechanisms in both alfalfa and barley plants. Succinate stimulated uptake by barley plants but had little effect on picloram absorption by alfalfa. As the concentration of sucrose was increased from 5 to 50 mM, uptake by alfalfa progressively increased such that at 50 mM concentration the total amount absorbed was

TABLE 4

The effect of various concentrations of succinate and sucrose on uptake of ^{14}C-picloram by intact roots of alfalfa and barley plants during a 4-hr treatment period

Substrate conc., mM	Root dpm[a]	% C[b]	Total dpm	% C	S/R[c]
Alfalfa					
Control	1,891	100	7,389 a	100	2.9
Succinate					
5	1,822	96	6,244 a	54	2.9
10	1,786	94	6,712 ab	90	2.8
50	1,710	90	5,847 a	79	2.4
Sucrose					
5	2,543	134	7,495 ab	101	1.9
10	3,305	174	8,477 bc	114	1.6
50	3,803	201	10,594 c	143	1.8
Barley					
Control	231	100	1,500 a	100	5.5
Succinate					
5	346	149	2,837 abc	189	7.2
10	341	147	2,483 abc	165	6.3
50	333	144	2,289 ab	152	5.9
Sucrose					
5	375	162	4,400 c	293	10.7
10	362	156	3,648 bc	243	9.0
50	452	195	3,255 abc	217	5.5

[a]Results are expressed as dpm per root or plant. Data presented are the means of 3 replicates. Means followed by the same letter are not significantly different from one another at the 5% level of probability as determined by Duncan's new multiple range test.

[b]Per cent of corresponding control.

[c]Ratio of the activity in the shoots to that in the roots.

approximately one and one-half times the control. Stimulation of uptake by barley plants, on the other hand, was greatest at 5 mM and decreased as the concentration of sucrose was increased. Similarly, 5 mM succinate enhanced uptake the most, although here also, higher concentrations up to 50 mM, still resulted in much greater uptake than by control plants. Total uptake by barley plants over a 4-hour period was increased three-fold in a 5 mM sucrose solution whereas the same concentration of succinate induced a two-fold increase.

The S/R ratios in Table 4 show that the presence of succinate did not alter the relative proportion of picloram in the roots and shoots of alfalfa. Sucrose, however, tended to reduce the proportion transported to the shoot. In barley plants low concentrations of succinate slightly promoted both uptake and transport. Similarly, uptake and transport were markedly enhanced by low concentrations of sucrose. At higher concentrations uptake was stimulated whereas transport was unaffected.

To determine whether plants were able to absorb ATP, roots of alfalfa and barley plants were placed in 100 ml of solution containing 1.0 μC of ^{14}C-8-ATP. The ATP concentration of the treatment solution was adjusted to 5 mM with unlabelled ATP. ATP molecules were assumed to be absorbed intact. Even if some or all of the ATP was broken down prior to entry into the plant, the absorption characteristics of the decomposition products would be expected to be essentially the same as those of ATP. The amount of radioactivity which was taken up by the plants was measured at various intervals and the corresponding amount of ATP was calculated in μg (Table 5).

TABLE 5

Uptake and translocation of ATP

by intact roots of alfalfa and barley plants over time[a]

Time, hr	Root µg ATP[b]	Total µg ATP	S/R[c]
Alfalfa			
1/2	5.2	7.5	0.4
1	9.0	11.8	0.3
2	14.4	19.4	0.4
4	27.0	38.3	0.4
8	42.9	58.2	0.4
12	85.1	117.0	0.4
Barley			
1/2	5.9	10.1	0.7
1	6.2	8.4	0.4
2	10.4	12.3	0.2
4	17.3	22.0	0.3
8	23.3	24.7	0.1
12	27.7	30.5	0.1

[a]Roots were placed in 100 ml of solution containing 1.0 µc of ^{14}C-8-ATP for 4 hr. Unlabelled ATP was added to the solution to increase the concentration to 5 mM.

[b]Results are expressed as µg of ATP per root or plant. Data presented are the means of 3 replicates.

[c]Ratio of the activity in the shoots to that in the roots.

Uptake of ATP by roots of intact alfalfa and barley plants continued for the duration of the 12-hour treatment period. Absorption was initially rapid and then proceeded at a nearly constant rate for the remainder of the experiment. Alfalfa plants and barley plants accumulated 117.0 and 30.5 µg of ATP, respectively, over 12 hours.

The ATP content of both roots and shoots of alfalfa increased proportionately over time, the S/R ratio remaining at 0.4. The amount of ATP in barley roots, on the other hand, increased over time whereas the amount transported to the shoots did not. Indeed, the S/R ratio decreased from 0.7 after a 1/2-hour exposure to the labelled solution to 0.1 after a 12-hour exposure.

Uptake of picloram by alfalfa and barley plants was not enhanced by the presence of either ADP or ATP in the treatment solution at concentrations ranging from 0.1 mM to 5.0 mM (Table 6). The highest concentrations of ATP reduced total accumulation of picloram by barley plants by nearly 40%. The S/R ratios presented in Table 6 indicate that ADP at all concentrations and ATP at the highest concentration used did not have any effect on the relative amount of picloram transported to the shoots of either alfalfa or barley. ATP at lower concentrations slightly depressed transport to alfalfa shoots but somewhat enhanced transport to barley shoots.

Pre-treatment of roots in a 5.0 mM ATP solution also failed to stimulate uptake (Table 7). Uptake by barley roots following pre-treatment in either ATP or ADP was, in fact, significantly decreased.

TABLE 6

The effect of various concentrations of ADP and ATP on uptake and translocation of ^{14}C-picloram by intact roots of alfalfa and barley plants during a 4-hr treatment period

Conc., mM	Root dpm[a]	% C[b]	Total dpm	% C	S/R[c]
Alfalfa					
Control	325	100	3,100 a	100	8.5
ADP					
0.1	242	74	3,274 a	105	12.5
1.0	329	101	3,350 a	108	9.2
5.0	292	90	3,227 a	104	10.0
ATP					
0.1	460	142	2,934 a	95	5.4
1.0	463	142	3,238 a	104	6.0
5.0	328	101	3,267 a	105	9.0
Barley					
Control	151	100	975 a	100	5.4
ADP					
0.1	105	70	900 ab	92	7.6
1.0	176	116	1,000 a	102	4.5
5.0	123	81	758 ab	77	5.2
ATP					
0.1	101	67	1,031 a	98	8.6
1.0	85	56	845 ab	87	8.9
5.0	94	62	616 b	63	5.6

[a]Results are expressed as dpm per root or plant. Data presented are means of 3 replicates. Means followed by the same letter are not significantly different from one another at the 5% level of probability as determined by Duncan's new multiple range test.

[b]Per cent of corresponding control.

[c]Ratio of the activity in the shoots to that in the roots.

TABLE 7

Uptake of ^{14}C-picloram during a 4-hr treatment period by intact and excised roots of alfalfa and barley plants pretreated with ADP (5 mM) or ATP (5 mM)[a]

Pretreatment	Intact		Excised
	Root dpm[b]	Total dpm	Root dpm
Alfalfa			
Control	4,910	14,284 a	4,813 a
ADP	6,858	18,757 a	4,848 a
ATP	4,856	14,192 a	4,177 a
Barley			
Control	211	762 a	113 a
ADP	55	451 b	79 ab
ATP	62	459 b	65 b

[a]Roots were pretreated for 4 hr in 10 ml of nutrient solution containing either ADP or ATP. Pretreatment conditions were identical to treatment conditions.

[b]Results are expressed as dpm per root or plant. Data presented are means of 3 replicates. Means followed by the same letter are not significantly different from one another at the 5% level of probability as determined by Duncan's new multiple range test.

These results support the view that externally supplied ATP exerts an inhibitory effect on the absorptive apparatus of plant cells (42, 102), and contradict reports that uptake of picloram and other nutrient ions by plant tissues is enhanced in the presence of ATP (32, 44, 87). Preliminary experiments revealed that the addition of ATP to unbuffered nutrient solutions reduced the pH to approximately 3.2, suggesting that enhanced uptake in the presence of ATP may be a direct result of lowered pH.

The Effect of Light

If 'active' picloram absorption is dependent on the utilization of high energy phosphate compounds derived from respiration, limited uptake would be expected if carbohydrate reserves in the plants are depleted by keeping the plants in the dark prior to and/or during treatment. Uptake by plants depleted of carbohydrate reserves might be expected to increase if respiratory substrates or ATP are provided in the culture solution. The results of experiments designed to show the effect of light and various energy sources on picloram uptake by plants exposed to different light regimes are given in Figures 15 and 16 for alfalfa and barley plants, respectively. Light regimes were divided into a pre-treatment period of either 10 hr in the light or 24 hr in the dark and a 4-hour treatment period either in light or dark.

Alfalfa plants subjected to the dark both prior to and during treatment (D/D) took up less than half as much as plants exposed to any of the other light regimes (L/L, L/D, D/L). Uptake by plants in the three light regimes with at least one light period was the same in each

Figure 15. The effect of various light regimes on uptake of ^{14}C-picloram by intact roots of alfalfa plants from nutrient solution containing 10 mM succinate (SN), 10 mM sucrose (SR) or 5 mM ATP. Results are expressed as dpm per root or plant.

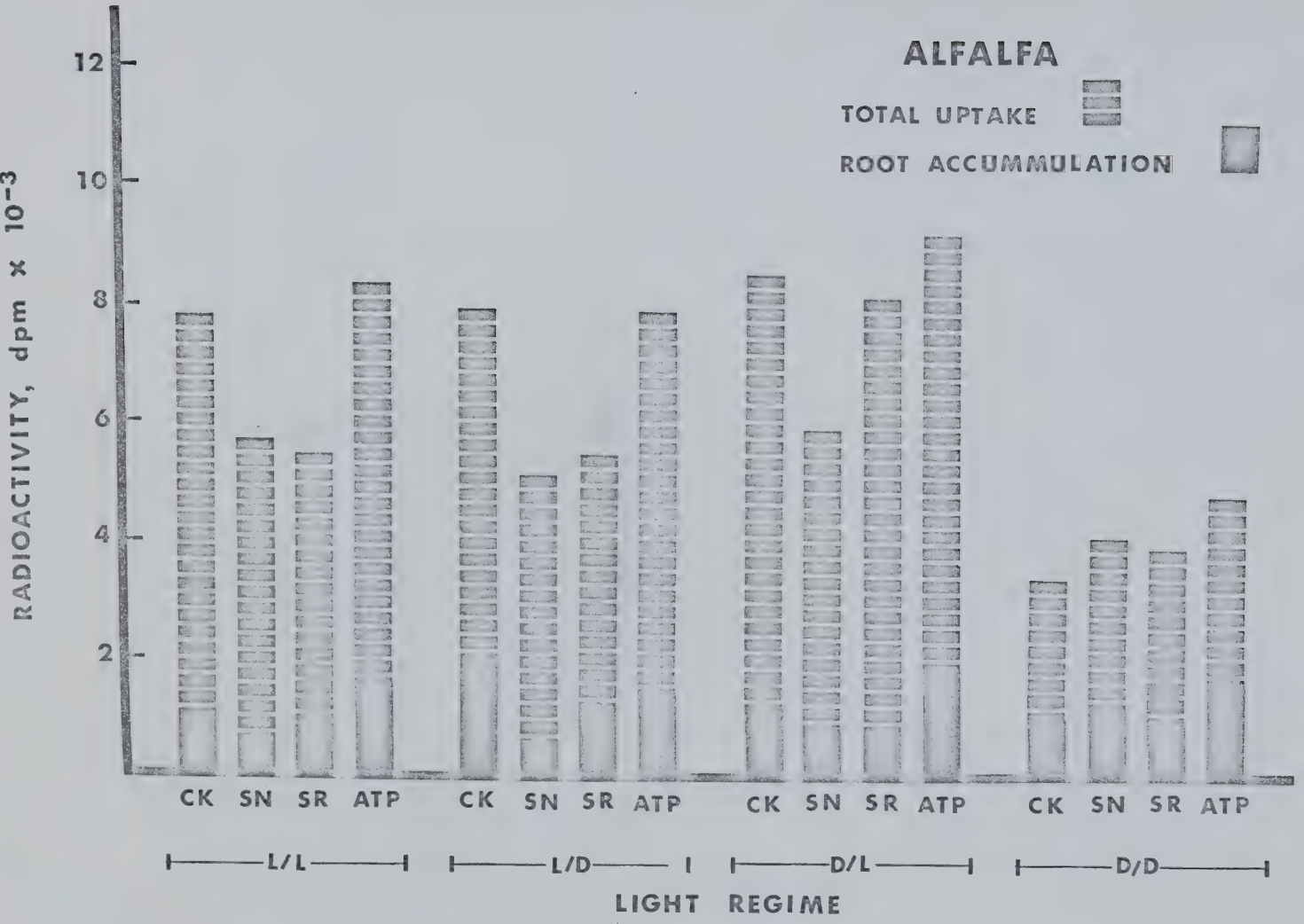
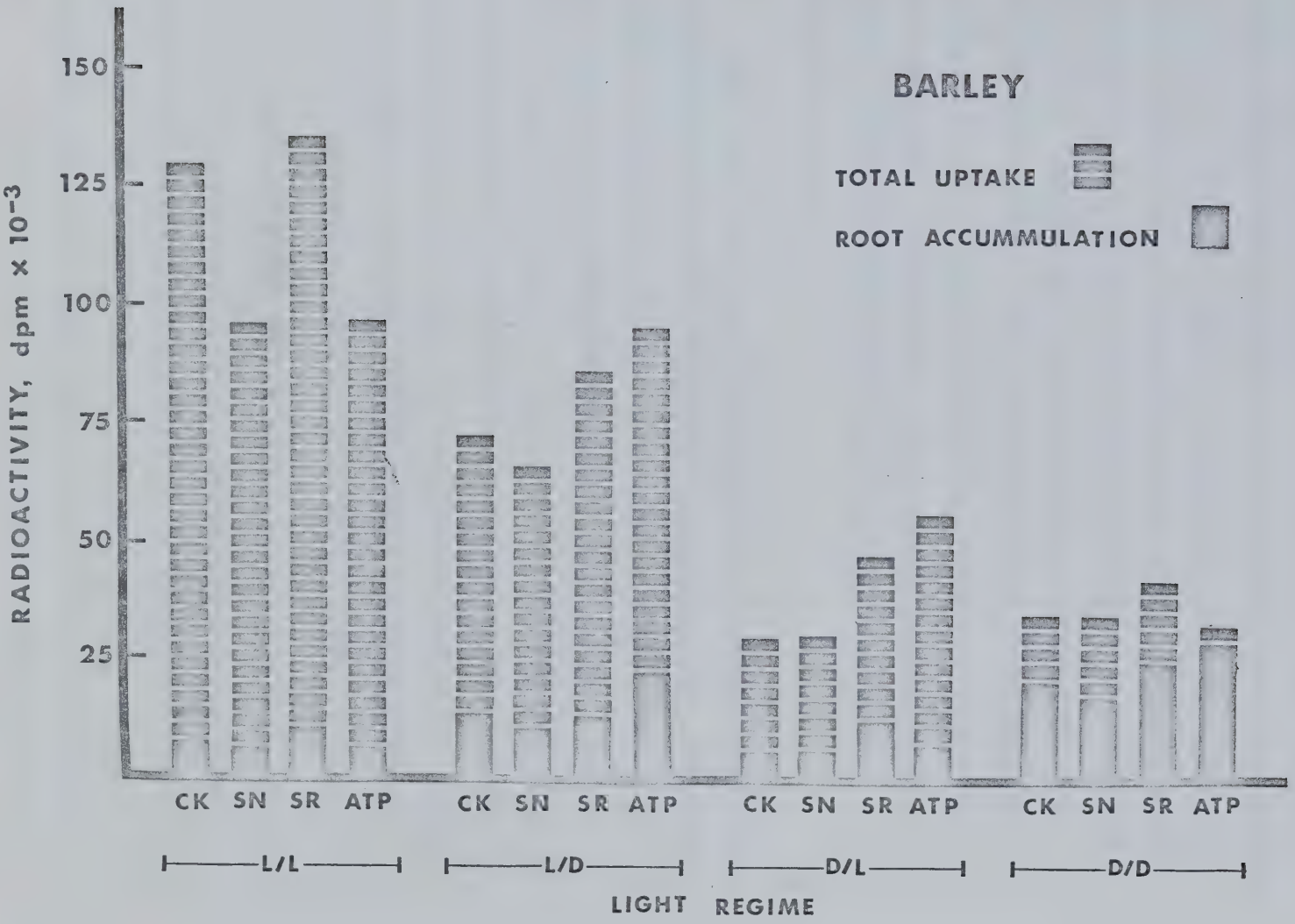

ALFALFA
TOTAL UPTAKE
ROOT ACCUMMULATION
RADIOACTIVITY, dpm x 10^{-3}
12
10
8
6
4
2
CK SN SR ATP CK SN SR ATP CK SN SR ATP CK SN SR ATP
L/L
L/D
D/L
D/D
LIGHT REGIME

Figure 16. The effect of various light regimes on uptake of ^{14}C-picloram by intact roots of barley plants from nutrient solution containing 10 mM succinate (SN), 10 mM sucrose (SR) or 5 mM ATP. Results are expressed as dpm per root or plant.


BARLEY
TOTAL UPTAKE
ROOT ACCUMMULATION
RADIOACTIVITY, dpm x 10⁻³
150
125
100
75
50
25
CK SN SR ATP
CK SN SR ATP
CK SN SR ATP
CK SN SR ATP
L/L
L/D
D/L
D/D
LIGHT REGIME

instance. Barley plants illuminated throughout the entire light regime (L/L), absorbed significantly more picloram at the 5% level of probability, than plants exposed to a period of darkness (L/D, D/L, D/D). Similarly, plants which were pre-illuminated but treated in the dark (L/D) took up significantly more picloram than plants which were not pre-illuminated (D/L, D/D). Picloram uptake by plants pretreated in the dark was not affected by the presence (D/L) or absence (D/D) of light during the uptake period. These results suggest that, in the dark, both alfalfa and barley plants become depleted of some readily available substrate possibly used in generating high energy compounds essential for picloram absorption.

Even though transpiration was reduced by dark treatment (the volume of treatment solution was measured after the uptake period), absorption by alfalfa plants exposed to L/D was as great as by those exposed to L/L, implying that uptake and transpiration were unrelated. Barley plants exposed to D/L took up no more picloram than those exposed to D/D, suggesting that, as with alfalfa, absorption and transpiration were not related. The reduced uptake by plants exposed to L/D compared to those exposed to L/L could be attributed to a rapid depletion of energy sources in barley plants in the absence of light.

Although total uptake of picloram by alfalfa plants which received no light (D/D) was markedly less than the amount absorbed by plants which received full illumination (L/L), accumulation in the roots of these plants did not differ appreciably. Conceivably, transport processes are more drastically affected by the exclusion of light than are root absorption mechanisms. The situation in barley plants may be

similar since roots of plants treated under dark conditions (D/D) accumulated three times as much picloram as roots of plants which received full illumination (L/L), even though total uptake by plants in the dark was only one-third as much as plants in the light. Barley plants pretreated in the dark took up no more picloram in the light (D/L) than in the dark (D/D), but the proportion of picloram transported to the shoots of the former more closely resembled the proportion transported to the shoots of plants which received full illumination (L/L). Shone and Wood (91) similarly reported that the relative rate of simazine movement in barley plants was affected by the light intensity during treatment even though total absorption was unaffected.

Neither succinate, sucrose nor ATP added to the treatment solution significantly enhanced total picloram absorption by alfalfa or barley plants. ATP did, however, appear to slightly stimulate uptake by alfalfa plants in all regimes except L/D. Both sucrose and ATP increased the amount absorbed by barley plants exposed to L/D and D/L regimes. In plants exposed to D/D, root accumulation increased slightly but total uptake was unchanged. Addition of succinate to the treatment solution caused a reduction in uptake by alfalfa plants exposed to L/L, L/D and D/L regimes and by barley plants exposed to the L/L and L/D regimes.

Plants exposed to different light regimes showed marked variation in the total amount of picloram absorbed. Conditions in the plants exposed to light either before or during treatment tended to favor increased absorption and/or translocation of picloram. Both sucrose and ATP appeared to partially substitute for the light requirement of

alfalfa. Similarly, ATP appeared to slightly compensate for reduced uptake by barley plants kept in the dark. These findings agree with the observations of Yamaguchi (110) who reported that plants depleted of carbohydrate reserves absorbed less 2,4-D than plants continually supplied with respiratory substrates through photosynthetic processes, and those of Sutcliffe (96) who stated that uptake of solutes by photosynthetic organisms kept in the dark was stimulated by an external energy supply.

Microautoradiographic Localization of ^{14}C-Picloram

Attempts to localize ^{14}C-picloram in the roots of alfalfa and barley plants were unsuccessful. No radioactivity was detected in the root sections whatever. An evaluation of the methods used in the investigation subsequently indicated probable reasons for the lack of success.

Most likely, insufficient ^{14}C-picloram was present in a 10 μ section to visibly darken the autoradiographic plates [20-30 grains per 25 μ^2 (101)] in an eight-week exposure. As a general rule, thin sections should be exposed to Kodak NTB No. 2 autoradiographic plates for a period ten times longer than that necessary to obtain a satisfactory image of whole tissues using medical X-ray film.* In gross autoradiographic investigations, X-ray films were exposed for 3 weeks, hence, in microautoradiographic studies, NTB plates should have been exposed for approximately 30 weeks. To decrease the exposure time, radiolabelled picloram with a much higher specific activity would have been required.

A distinct possibility also exists that the ^{14}C-picloram which

*Dr. P. R. Gorham - Personal Communication

accumulated in the roots was displaced during the infiltration process. The solubility of picloram in paraffin is unknown, however, the compound is known to be sparingly soluble in organic solvents such as benzene (200 ppm) and diethyl ether (1,200 ppm). Since the root segments were in molten paraffin for two to three weeks, the ^{14}C-picloram initially present in the root could well have diffused out. Alternative procedures described by Fisher and Housley (29) and others (80, 81) for autoradiography of diffusible substances may have provided better results.

DISCUSSION AND CONCLUSIONS

Although explanations concerning the outcome of particular experiments were given in the previous section, the results must be examined collectively in order to draw meaningful conclusions.

At pH values less than the pK of picloram, uptake of the acid was substantially increased (Figure 1). Increased absorption of 2,4-D at low pH has been attributed to the fact that unionized, uncharged molecules approach the negatively charged cellulosic and cell membrane surfaces more readily than do the negatively charged dissociated ions (22, 110). Isensee *et al.* (45) contended that picloram, like 2,4-D, is preferentially absorbed in the undissociated molecular form. Such a contention presupposes that the undissociated molecules pass through the lipid-rich portions of the plasmalemma. Recent evidence, however, indicated that the uptake of organic acids occurs chiefly in the anionic form through hydrophilic regions of the limiting membrane (46). Undoubtedly, at pH values normally encountered in the soil, uptake of picloram is confined largely to the anionic form.

The upper limit of the physiological range of H^+-ion concentration is considered to be approximately 10^{-4}M. The possibility that enhanced uptake at higher concentrations may have been due to an alteration of the metabolic activity or structural integrity of the root cannot be precluded. The decline in uptake as pH was increased actually may have been due to changes in the ionization of absorptive root surfaces rather than in the availability of the undissociated molecules.

The results of the present study cast doubt on the conclusion drawn by Baur and Bovey (5) that a large decrease in picloram uptake at high pH values can be ascribed to the degradation of binding sites by the enzyme phospholipase D, which shows maximum activity at pH 5.5. If phopholipase D has an effect on uptake mechanisms, increased absorption would be expected at pH values greater than 5.5 since the optimal pH for enzyme activity would be surpassed. Uptake at pH 7.0 was, in fact, essentially the same as at pH 5.5 (Figure 1). Furthermore, instability of absorptive sites at pH values normally considered favorable for plant growth seems highly unlikely.

Autoradiographic studies showed that picloram was rapidly absorbed from culture solution by roots of both alfalfa and barley plants. Alfalfa root tips tended to accumulate more picloram than did other regions of the root, whereas barley roots accumulated essentially the same amount throughout. On the one hand, picloram may have been preferentially absorbed by alfalfa root tips. On the other hand, transport away from the tips may have been restricted for lack of sufficiently developed xylem vessels. Alternatively, a certain amount of picloram taken up by other portions of the root may have been transported to the actively growing root apices. Accumulation of dicamba, another auxinic herbicide, in root tips of Tartary buckwheat plants following foliar application, and basipetal movement of 2,4-D in barley roots have been reported (12, 22).

Translocation of root-absorbed picloram to alfalfa and barley shoots also was very rapid. Substantial amounts of the herbicide were transported to the shoots within 2 to 4 hr, accumulating predominantly

in the meristematic regions. These results are consistant with the findings of Reid and Hurtt (78) who observed preferential accumulation of root-absorbed picloram in the apical regions of bean shoots. Chang (12) noted a similar distribution of dicamba in Tartary buckwheat plants following root uptake. Appreciable amounts of picloram may first be transported to the mature leaves via the xylem vessels, whereupon it is readily redistributed to the assimilate stream and ultimately concentrated in the meristematic regions.

Under similar treatment conditions, the total amount of picloram taken up by alfalfa plants was generally much greater than the amount absorbed by barley plants of nearly equivalent mass. Conceivably, differential absorption partly accounts for the difference in the inherent susceptibility of alfalfa (very sensitive) and barley (resistant) to picloram.

Time course studies of picloram uptake from culture solution by roots of alfalfa and barley plants indicated that absorption occurs in two separate phases, an initial phase of rapid uptake followed by a brief lag period and a phase of continuing absorption over an extended period. Initial uptake of picloram by roots of both species seems to be governed by passive processes, whereas continuing absorption appears to be controlled, at least in part, by active mechanisms. The lag period conceivably represents the interval between the time a physical equilibrium is attained in the roots and the initiation of metabolically mediated uptake processes.

Initial entry of picloram into roots appears to be largely by

diffusion, although a small amount may be adsorbed in an exchangeable form within the root. The rapid release of a large portion of picloram in roots following transfer from the uptake solution to unlabelled nutrient solution (Figure 6, Table 1) substantiates this conclusion. Presumably, picloram simply diffused out of the intercellular spaces of the root cortical tissue. That which remained in the roots for periods exceeding 4 hr was either tenaciously bound in a non-exchangeable form or retained behind a physiological barrier pending transport to the shoots. In this respect picloram uptake is not analogous to absorption of 2,4-D, a large proportion of which is adsorbed in an exchangeable fraction in roots, particularly on the epidermis (22, 50).

Although a hyberbolic relationship between uptake and external concentration has frequently been considered indicative of either physical adsorption to non-specific binding sites or active absorption by carrier-mediated processes, the absence of such a relationship does not obviate the possibility that one or the other or both of the mechanisms are operative. Concentrations of picloram necessary to saturate adsorptive sites may be considerably greater than 20 μM, the highest concentration used in this investigation. Indeed, Glass and Bohm (35) showed that saturation kinetics were not apparent for uptake of arbutin, a simple phenolic compound, by barley roots until concentrations in the external solution exceeded 5 mM.

Time course studies of picloram absorption in the presence of an inhibitor (1 mM NaN_3) showed that active uptake mechanisms were operative in alfalfa roots after 1 hr and in barley roots after 2 hr in treatment solution (Figures 13 and 14). Q_{10} values greater than

2 were recorded for the uptake of picloram over longer periods (Table 3), further establishing that metabolic processes are involved in the phase of continuing absorption.

The metabolic component of uptake was either depressed or eliminated by a number of respiratory inhibitors including DNP, NaN_3, and oligomycin (Figures 9 and 10). Increasing concentrations of all three inhibitors had the same effect, namely reducing picloram uptake by both alfalfa and barley plants. DNP was most effective, followed by NaN_3 and oligomycin, respectively. Differences between the effects of the inhibitors on uptake of picloram can be attributed, at least in part, to the properties of the inhibitor. In the presence of DNP at inhibitory concentrations, the hydrolysis of high energy compounds is promoted and the synthesis of ATP abolished. Sodium azide, on the other hand, inhibits phosphorylation but does not bring about the hydrolysis of ATP. Oligomycin inhibits only the conversion of high energy intermediates to ATP.

Decidedly, respiration is essential for the active uptake of picloram into roots through the production of high energy phosphate molecules such as ATP which are capable of driving active uptake mechanisms. Greater absorption of picloram in the presence of NaN_3 or oligomycin than in the presence of DNP can be attributed to utilization of 'residual' ATP which was sufficient to sustain active uptake for a time after the plants were placed in the treatment solution. Furthermore, uptake processes apparently can be driven by high energy compounds other than ATP since uptake was greater in the presence of oligomycin than in the presence of NaN_3. Uptake does not appear to

be linked to electron transport processes since NaN_3 was less effective than DNP in inhibiting uptake. Active absorption of picloram is most probably directly affiliated with the utilization of high energy compounds which are the products of oxidative phosphorylation. A greater decrease in picloram uptake by alfalfa plants than by barley plants exposed to 1 mM DNP in the concentration series (Figure 9) and to 1 mM NaN_3 in the time course studies (Figures 13 and 14) suggests that absorption by the former is more dependent on a supply of metabolic energy than that by the latter.

The differential effect of cycloheximide and chloramphenicol on uptake of picloram by alfalfa and barley plants cannot be explained readily. The dissimilar response to increasing concentrations of the inhibitors suggests, however, that different respiratory pathways may exist in the roots of the two species. Increased total uptake of picloram by alfalfa plants in the presence of chloramphenicol (Figure 11) may be analogous to similar increases observed in plants exposed to low concentrations of DNP and NaN_3 (Figures 9 and 10). Although total uptake was doubled in 5 mM chloramphenicol solution, the amount transported to the shoots was approximately the same as in control plants.

Reduced picloram absorption by alfalfa plants which were kept in the dark both prior to and during treatment and barley plants kept in the dark either prior to or during treatment indicated a close link between picloram uptake and photosynthetic reactions. Presumably, enhanced uptake and transport of picloram were effected through mechanisms which are dependent on energy derived from carbohydrates

and other energy substrates formed during photosynthesis.

The effect of sucrose added to the treatment solution was to greatly increase total picloram absorption by both alfalfa and barley plants except in experiments in which plants were exposed to different light regimes, in which case reduced absorption may have been due to an osmotic imbalance created by a rather high sucrose concentration (50 mM) in the external solution. Under natural conditions, sucrose is widely distributed in plants and, in most instances, is the principal form in which carbohydrates are translocated. Sucrose is, then, a readily available substrate for respiration. Its breakdown in the glycolytic pathway and Krebs' tricarboxylic acid (TCA) cycle yields large quantities of ATP which more than likely drive uptake mechanisms.

Succinate, an organic acid constituent of the TCA cycle, enhanced uptake by barley plants but had little effect on absorption by alfalfa plants except in the light regime studies, in which succinate depressed uptake by both species. Increased uptake could be attributed to increased synthesis of ATP as a result of increased availability of succinate. Current evidence, however, suggests that succinate may have a more direct effect on the permeability status of cell membranes (46). Enhanced uptake by barley roots at low succinate concentrations (5 mM) and depressed uptake by alfalfa and barley roots at high concentrations (50 mM) may be related to changes in membrane permeability.

Neither pretreatment of roots with ATP nor addition of ATP to the treatment solution significantly stimulated absorption of picloram by alfalfa or barley plants. Uptake by barley roots was, in fact,

markedly depressed. These results contrast with findings reported elsewhere that uptake of picloram by soybean hypocotyl and barley coleoptile sections was virtually doubled in the presence of 4 mM ATP (86). Increased uptake which has been attributed to the influence of ATP on uptake mechanisms may actually have been due to a high H^+-ion concentration in inadequately buffered solutions. Preliminary experiments revealed that a substantial increase in picloram uptake was brought about by addition of ADP or ATP to unbuffered treatment solutions.

Inhibition of ion uptake by barley roots in the presence of ATP has been attributed to reduced activity of respiratory chain enzymes located in the mitochondria (63). Vakhmistrov and Listova (102) have suggested, however, that externally applied ATP causes a spatial disruption of cell membrane structure in roots. Quite probably, the rather large and metabolically active ADP and ATP molecules are unable to pass into the cortical cells of the root without interfering with uptake mechanisms operating in the plasmalemma.

In some instances, ATP appeared to slightly stimulate uptake of picloram by plants exposed to a dark period either before or during treatment. On the basis of the foregoing conclusion, however, it is unlikely that ATP actually affected the intracellular metabolism or uptake mechanisms located in the plasmalemma.

The effect of ADP on uptake was essentially the same as the effect of ATP. Jacoby (47) has suggested that ATP added to a treatment solution is partly decomposed to ADP, and that ADP and ion transport compete for a common high energy intermediate. That solute uptake and

ATP formation are antagonistic seems highly unlikely in view of the fact that metabolically mediated uptake processes are generally considered to be driven by energy supplied by ATP. Quite likely, the similar effects of ADP and ATP can be ascribed to their closely related chemical structures.

Summary

This investigation has shown that the uptake and translocation of picloram by intact roots of alfalfa and barley plants involves a strong physical diffusion component as well as associated metabolic processes. The relative importance of the two means of entry and of movement within plants apparently varies between species.

Time course studies indicate that alfalfa and barley have similar absorption patterns consisting of a rapid initial uptake lasting for 1 to 2 hr, followed by a slower continuing absorption occurring at a nearly constant rate for the duration of a 12-hour uptake period. The pH of the external medium has a marked influence on the amount of picloram absorbed during the continuing phase. Total accumulation of picloram is markedly greater in alfalfa plants (susceptible) than in barley plants (tolerant) of similar mass, which may account, at least in part, for the difference in susceptibility of the two species.

Initial absorption of picloram from culture solution by either species is not reduced by respiratory inhibitors and is not temperature-dependent, indicating that this phase of absorption involves primarily passive processes. Continuing absorption of picloram, on the other hand, is temperature-dependent and is drastically reduced by metabolic

inhibitors. Uptake during this phase is enhanced by the presence of respiratory substrates in the treatment solution.

The results of inhibitor studies suggest that picloram absorption by alfalfa plants is more dependent on a supply of metabolic energy than is uptake by barley plants. Both alfalfa and barley plants, however, show similar reductions in uptake when they are deprived of a source of light, indicating that, in both species, adequate supply of carbohydrate reserves is necessary to drive uptake mechanisms. Externally applied ATP does not stimulate absorption.

LITERATURE CITED

1. Ahmad, M. and M. Cailloux. 1972. Effects of some respiratory inhibitors on water flux in root hairs of *Avena sativa*. Can. J. Bot. 50: 575-580.

2. Arisz, W. H. 1956. Significance of the symplasm theory for transport across the root. Protoplasma 46: 5-62.

3. Bachelard, E. P. and R. D. Ayling. 1971. The effects of picloram and 2,4-D on plant cell membranes. Weed Res. 11: 31-36.

4. Baker, D. A. 1971. Barriers to the radial diffusion of ions in maize roots. Planta 98: 285-293.

5. Baur, J. R. and R. W. Bovey. 1969. Distribution of root-absorbed picloram. Weed Sci. 17: 524-528.

6. Baur, J. R. and R. W. Bovey. 1970. The uptake of picloram by potato tuber tissue. Weed Sci. 18: 22-24.

7. Biddulph, S. F. 1967. A microautoradiographic study of Ca^{45} and S^{35} distribution in the intact bean root. Planta 74: 350-367.

8. Blackman, G. E. and J. A. Sargent. 1959. The uptake of growth substances. II. The absorption and accumulation of 2,3,5-triiodobenzoic acid by the root and frond of *Lemna minor*. J. Exp. Bot. 10: 480-503.

9. Bowling. D. J. F. 1966. Active transport of ions across sunflower roots. Planta 69: 377-382.

10. Branton, D. and L. Jacobson. 1962. Iron localization in pea plants. Plant Physiol. 37: 546-551.

11. Brouwer, R. 1965. Ion absorption and transport in plants. Ann. Rev. Plant Physiol. 16: 241-266.

12. Chang, F. Y. 1969. Translocation and metabolism of 2-methoxy-3,6-dichlorobenzoic acid in plants. Ph.D. Dissert. Univ. of Alberta, Edmonton.

13. Chang, I. K. and C. L. Foy. 1971. Effects of picloram on mitochondrial swelling and ATPase. Weed Sci. 19: 54-58.

14. Christie, A. E. and A. C. Leopold. 1965. Entry and exit of indoleacetic acid in corn coleoptiles. Plant and Cell Physiol. 6: 453-465.

15. Clarkson, D. T., A. W. Robards, and J. Sanderson. 1971. The tertiary endodermis in barley roots: Fine structure in relation to radial transport of ions and water. Planta 96: 292-305.

16. Clarkson, D. T. and J. Sanderson. 1970. Relationship between the anatomy of cereal roots and the absorption of nutrients and water. Agricultural Research Council Letcombe Laboratory Annual Report 1970. pp. 16-25.

17. Clarkson, David T., J. Sanderson and R. S. Russell. 1968. Ion uptake and root age. Nature 220: 805-806.

18. Corbin, F. T., R. P. Upchurch and F. L. Selman. 1971. Influence of pH on the phytotoxicity of herbicides in soil. Weed Sci. 19: 233-239.

19. Crafts, A. S. and T. C. Broyer. 1938. Migration of salts and water into xylem of the roots of higher plants. Am. J. Botany 25: 529-535.

20. Crafts, A. S. and S. Yamaguchi. 1960. Absorption of herbicides by roots. Am. J. Bot. 47: 248-255.

21. Crafts, A. S. and S. Yamaguchi. 1964. The Autoradiography of Plant Materials. University of California Agr. Publ., Manual 35. Berkeley, Calif. 143 p.

22. Donaldson, T. W. 1967. Absorption of the herbicides 2,4-D and monuron by barley roots. Ph.D. Dissert. Univ. California, Davis.

23. Dumbroff, E. B. and D. R. Peirson. 1971. Probable sites for passive movement of ions across the endodermis. Can. J. Bot. 49: 35-38.

24. Eisinger, W. R., and D. J. Morre. 1971. Growth-regulating properties of picloram, 4-amino-3,5,6-trichloropicolinic acid. Can. J. Bot. 49: 889-897.

25. Ellis, R. J. and I. R. MacDonald. 1970. Specificity of cycloheximide in higher plant systems. Plant Physiol. 46: 227-232.

26. Epstein, E. 1956. Mineral nutrition of plants: Mechanisms of uptake and transport. Ann. Rev. Plant Physiol. 7: 1-21.

27. Epstein, E. E. 1972. Mineral Nutrition of Plants: Principles and Perspectives. John Wiley and Sons, Inc., New York. 412 p.

28. Fisher, D. A., D. E. Bayer, and T. E. Weier. 1968. Morphological and anatomical effects of picloram on *Phaseolus vulgaris*. Bot. Gaz. 129: 67-70.

29. Fisher, D. B. and T. L. Housley. 1972. The retention of water-soluble compounds during freeze-substitution and micro-autoradiography. Plant Physiol. 49: 166-171.

30. Fisher, J. D., Dale Hansen, and T. K. Hodges. 1970. Correlation between ion fluxes and ion-stimulated adenosine triphosphatase activity of plant roots. Plant Physiol. 46: 8;2-814.

31. Fisher, J. and T. K. Hodges. 1969. Monovalent ion stimulated adenosine triphosphatase from oat roots. Plant Physiol. 44: 385-395.

32. Francki, R. I. B., M. Zaitlin and R. G. Jensen. 1971. Metabolism of separated leaf cells. II. Uptake and incorporation of protein and ribonucleic acid precursors by tobacco cells. Plant Physiol. 48: 14-18.

33. Frank, R. and C. M. Switzer. 1969. Absorption and translocation of pyrazon by plants. Weed Sci. 17: 365-370.

34. Ginsburg, H. and B. Z. Ginsburg. 1970. Radial water and solute flows in roots of *Zea mays*. II. Ion fluxes across root cortex. J. Exp. Bot. 21: 593-604.

35. Glass, A. D. M. and B. A. Bohm. 1971. The uptake of simple phenols by barley roots. Planta 100: 93-105.

36. Gruener, N. and J. Neumann. 1966. An ion-stimulated adenosine triphosphatase from bean roots. Physiol. Plant. 19: 678-682.

37. Hall, J. L. 1969. Localization of cell surface adenosine triphosphatase activity in maize roots. Planta 85: 105-107.

38. Hall, J. L. 1970. Pinocytotic vesicles and ion transport in plant cells. Nature 226: 1253-1254.

39. Hamaker, J. W., H. Johnston, R. T. Martin and C. T. Redemann. 1963. A picolinic acid derivative: A plant growth regulator. Science 141: 363.

40. Higinbotham, N. 1959. The possible role of ATP in rubidium absorption as revealed by the influence of external phosphate, DNP and arsenate. Plant Physiol. 34: 645-650.

41. Hoagland, D. R. and D. I. Arnon. 1950. The water-culture method for growing plants without soil. Calif. Agric. Expt. Sta. Circ. 347, 32 p.

42. Hodges, T. K. 1966. Oligomycin inhibition of ion transport in plant roots. Nature 209: 425-426.

43. Hodges, T. K. and O. E. Elzam. 1967. Effect of azide and oligomycin on the transport of calcium ions in corn mitochondria. Nature 215: 970-972.

44. Hodges, T. K. and J. B. Hanson. 1965. Calcium accumulation by maize mitochondria. Plant Physiol. 40: 101-109.

45. Isensee, A. R., G. E. Jones and B. C. Turner. 1971. Root absorption and translocation of picloram by oats and soybeans. Weed Sci. 19: 727-731.

46. Jackson, P. C., J. M. Taylor, and S. B. Hendricks. 1970. Entry of organic acid anions into roots. Proc. Nat. Acad. Sci. 65: 176-183.

47. Jacoby, Benjamin. 1965. The effect of ATP on uptake of monovalent cations and anions by red beet slices. J. Exp. Bot. 16: 243-248.

48. Jacoby, B. and O. E. Plessner. 1970. Oligomycin effect on ion absorption by excised barley roots and on their ATP content. Planta 90: 215-221.

49. Jensen, Gustav. 1962. Active and passive components in ion uptake processes. Physiol. Plant. 15: 363-368.

50. Johnson, M. P. and James Bonner. 1956. The uptake of auxin by plant tissue. Physiol. Plant. 9: 102-118.

51. Jones, P. C. T. 1970. The effect of light, temperature, and anaesthetics on ATP levels in the leaves of *Chenopodium rubrum* and *Phaseolus vulgaris*. J. Exp. Bot. 21: 58-63.

52. Kefford, N. P. and O. H. Caso. 1966. A potent auxin with unique chemical structure — 4-amino-3,5,6-trichloropicolinic acid. Bot. Gaz. 127: 159-163.

53. Laties, G. G. 959. Active transport of salt into plant tissues. Ann. Rev. Plant Physiol. 10: 87-112.

54. Laties, G. G. and K. Budd. 964. The development of differential permeability in isolated steles of corn roots. Proc. Natl. Acad. Sci. 52: 462-469.

55. Lauger, P. 1972. Carrier-mediated ion transport. Science 178: 24-30.

56. Leggett, J. E. 1968. Salt absorption by plants. Ann. Rev. Plant Physiol. 19: 333-346.

57. Leonard, R. T. and J. B. Hanson. 1972. Increased membrane-bound adenosine triphosphatase activity accompanying development of enhanced solute uptake in washed corn root tissue. Plant Physiol. 49: 436-440.

58. Lundegardh. H. 1955. Mechanisms of absorption, transport, accumulation, and secretion of ions. Ann. Rev. Plant Physiol. 6: 1-24.

59. Luttge, Ulrich and George G. Laties. 1967. Selective inhibition of absorption and long distance transport in relation to the dual mechanisms of ion absorption in maize seedlings. Plant Physiol. 42: 181-185.

60. Maas, E. V. and Gen Ogata. 1971. Absorption of magnesium and chloride by excised corn roots. Plant Physiol. 47: 357-360.

61. MacRobbie, E. A. C. 1965. The nature of coupling between light energy and active ion transport in *Nitella translucens*. Biochim. Biophys. Acta 94: 64-73.

62. Meikle, R. W., E. A. Williams, and C. T. Redemann. 1966. Metabolism of Tordon herbicide (4-amino-3,5,6-trichloro-picolinic acid) in cotton and decomposition in soil. J. Agr. Food Chem. 14: 384-387.

63. Mengel, K. 1963. Der Einfluss von ATP-Zugaben und weiterer Stoffwechselagenzien auf die Rb-Aufnahme abgeschnittener Gerstenwurzeln. Physiol. Plant. 16: 767-776.

64. Moody, K., C. A. Kust and K. P. Buchholtz. 1970. Release of herbicides by soybean roots in culture solutions. Weed Sci. 18: 214-218.

65. Moody, K., C. A. Kust, and K. P. Buchholtz. 1970. Uptake of herbicides by soybean roots in culture solutions. Weed Sci. 18: 642-647.

66. Muzik, T. J. 1969. Effect of temperature on uptake of herbicides by wheat roots. Abstr. Meet. Weed Sci. Soc. Am. 16.

67. Nalewaja, John D., Richard Behrens and A. R. Schmid. 1964. Uptake, translocation and fate of EPTC-C^{14} in alfalfa. Weeds 12: 269-272.

68. Nashed, R. B. and R. D. Ilnicki. 1970. Absorption, distribution, and metabolism of linuron in corn, soybean, and crabgrass. Weed Sci. 18: 25-28.

69. Oberlander, H. E. 1966. The relative importance of active and passive processes in ion translocation across root tissue. In: Limiting steps in ion uptake by plants from soil. Int. Atomic Energy Agency, Vienna. Tech. Rep. Ser. No. 65: 101-120.

70. Perkins, H. J., C. D. Nelson and P. R. Gorham. 1959. A tissue-autoradiographic study of the translocation of C^{14}-labelled sugars in the stems of young soybean plants. Can. J. Bot. 37: 891-877.

71. Pitman, M. G. 1972. Uptake and transport of ions in barley seedlings. II. Evidence for two active stages in transport to the shoot. Aust. J. Biol. Sci. 25: 243-257.

72. Poole, Ronald J. and K. V. Thimann. 1964. Uptake of indole-3-acetic acid and indole-3-acetonitrile by *Avena* coleoptile sections. Plant Physiol. 39: 98-103.

73. Prasad, R. and G. E. Blackman. 1962. Factors affecting the uptake of 2,2-dichloropropionic acid by roots and fronds of *Lemna minor*. Plant Physiol. 37: xiii-xiv.

74. Rains, D. W., W. E. Schmid and Emanuel Epstein. 1964. Absorption of cations by roots. Effects of hydrogen ions and essential role of calcium. Plant Physiol. 39: 274-278.

75. Rajan, A. K. 1966. The effect of root temperatures on water and sulphate absorption in intact sunflower plants. J. Exp. Bot. 17: 1-18.

76. Rathore, V. S., S. H. Wittwer, W. H. Jyung, Y. P. S. Bajaj and M. W. Adams. 1970. Mechanism of zinc uptake in bean (*Phaseolus vulgaris*) tissues. Physiol. Plant. 23: 908-919.

77. Reid, C. P. P. and W. Hurtt. 1969. Translocation and distribution of picloram in bean plants associated with nastic movements. Plant Physiol. 44: 1393-1396.

78. Reid, C. P. P. and W. Hurtt. 1970. Root permeability as affected by picloram and other chemicals. Physiol. Plant. 23: 124-130.

79. Reinhold, Leonora. 1954. The uptake of indole-3-acetic acid by pea epicotyl segments and carrot disks. New Phytologist 53: 217-239.

80. Rogers, A. W. 1967. Techniques of Autoradiography. Elsevier Publishing Company, Amsterdam. 335 p.

81. Roth, L. J. and W. E. Stumpf. 1969. Autoradiography of Diffusible Substances. Academic Press, Inc., London. 371 p.

82. Saunders, P. F., C. F. Jenner and G. E. Blackman. 1966. The uptake of growth substances. VI. A comparative study of the factors determining the patterns of uptake of phenoxyacetic acid and 2,4,5-trichloroephenoxyacetic acid, weak and strong auxins, by *Gossypium* tissues. J. Exp. Bot. 17: 241-269.

83. Salisbury, F. B. and C. Ross. 1969. Plant Physiology. Wadsworth Publishing Company, Inc., Belmont, Calif. 747 p.

84. Scott, P. C. and R. O. Morris. 1970. Quantitative distribution and metabolism of auxin herbicides in roots. Plant Physiol. 46: 680-684.

85. Sexton, R. and J. F. Sutcliffe. 1969. Some observations on the characteristics and distribution of adenosine triphosphatases in young roots of *Pisum sativum*, cultivar Alaska. Ann. Bot. 33: 683-694.

86. Sharma, M. P. 1971. Physiological and biochemical studies on picloram action in plants. Ph.D. Dissert. Univ. Alberta, Edmonton.

87. Sharma, M. P. and W. H. Vanden Born. 1972. Effects of picloram on growth and on chlorophyll, RNA and protein content of plants. Can. J. Botany 50: 2039-2044.

88. Sheets, T. J. 1961. Uptake and distribution of simazine by oat and cotton seedlings. Weeds 9: 1-13.

89. Shimabukuro, R. H. and A. J. Linck. 1967. Root absorption and translocation of atrazine in oats. Weeds 15: 175-178.

90. Shone, M. G. T. and D. A. Barber. 1966. The initial uptake of ions by barley roots. I. Uptake of anions. J. Exp. Bot. 17: 78-88.

91. Shone, M. G. T. and Ann V. Wood. 1972. Factors affecting absorption and translocation of simazine by barley. J. Exp. Bot. 23: 141-151.

92. Sikka, H. C. and D. E. Davis. 1968. Absorption, translocation and metabolism of prometryne in cotton and soybean. Weed Science 16: 474-477.

93. Slater, E. C. 1963. Uncouplers and inhibitors of oxidative phosphorylation. In: Metabolic Inhibitors, Vol. II. R. M. Hochster and J. H. Quastel, eds. Academic Press, London. p. 503-503.

94. Strang, R. H. and R. L. Rogers. 1971. A microradioautographic study of ^{14}C-diuron absorption by cotton. Weed Sci. 19: 355-362.

95. Strang, R. H. and R. L. Rogers. 1971. A microradioautographic study of ^{14}C-trifluralin absorption. Weed Sci. 19: 363-369.

96. Sutcliffe, J. F. 1962. Mineral Salts Absorption in Plants. Pergamon Press, New York. 194 p.

97. Swanson, C. R. and J. R. Baur. 1969. Absorption and penetration of picloram in potato tuber discs. Weed Sci. 17: 311-314.

98. Swets, W. A. and R. T. Wedding. 1964. The effect of DNP and other inhibitors on the uptake and metabolism of 2,4-D by Chlorella. New Phytologist 63: 55-72.

99. Tames, R. S. and R. J. Hance. 1969. The adsorption of herbicides by roots. Plant and Soil 30(2): 221-226.

100. Taylor, T. D. and G. F. Warren. 1970. The effect of metabolic inhibitors on herbicide movement in plants. Weed Sci. 18: 68-74.

101. Trip, P. and P. R. Gorham. 1968. Bidirectional translocation of sugars in sieve tubes of squash plants. Plant Physiol. 43: 877-882.

102. Vakhmistrov, D. B. and M. P. Listova. 1967. Direct action of ATP on the absorptive apparatus of barley roots. Sov. Plant Phys. 14: 700-707.

103. Venis, M. A. and G. E. Blackman. 1966. The uptake of growth substances. VIII. Accumulation of chlorinated benzoic acids by *Avena* segments: A possible mechanism for the transient phase of accumulation. J. Exp. Bot. 17: 771-789.

104. Venis, M. A. and G. E. Blackman. 1966. The uptake of growth substances. IX. Further studies on the mechanism of uptake of 2,3,6-trichlorobenzoic acid by *Avena* segments. J. Exp. Bot. 17: 790-808.

105. Von Guttenberg, H. and K. Zetsche. 1956. Der Einfluss des Lichtes auf die Auxinbildung und den Auxintransport. Planta 48: 99-134.

106. Vostral, H. J., K. P. Buchholtz and C. A. Kust. 1970. Effect of root temperature on absorption and translocation of atrazine in soybeans. Weed Sci. 18: 115-117.

107. Wax, L. M. and R. Behrens. 1965. Absorption and translocation of atrazine in quackgrass. Weeds 13: 107-109.

108. Webb, J. A. and P. R. Gorham. 1964. Translocation of photosynthetically assimilated C^{14} in straight-necked squash. Plant Physiol. 39: 663-672.

109. Wojtaszek, T. 1966. Relationship between susceptibility of plants to DNP and their capacity for ATP generation. Weeds 14(2): 125-129.

110. Yamaguchi, S. 1965. Analysis of 2,4-D transport. Hilgardia 36: 349-378.

111. Yu, G. H. and P. J. Kramer. 1967. Radial salt transport in corn roots. Plant Physiol. 42: 985-990.

112. Yu, G. H. and P. J. Kramer. 1969. Radial transport of ions in roots. Plant Physiol. 44: 1095-1100.